(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 June 2003 (19.06.2003)

PCT

(10) International Publication Number
WO 03/049723 A2

(51) International Patent Classification[7]: A61K 31/00, 38/00, G01N 33/50, C12Q 1/37, A61P 11/06, 17/00, 19/02, 29/00, 37/06, 37/08

(21) International Application Number: PCT/GB02/05658

(22) International Filing Date:
13 December 2002 (13.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0129848.8	13 December 2001 (13.12.2001)	GB
0131103.4	31 December 2001 (31.12.2001)	GB

(71) Applicant *(for all designated States except US)*: **KOWA COMPANY LTD** [JP/JP]; 6-29 Nishiki 3-chome, Naka-ku, Nagoya-shi, Aichi-ken 460-8625 (JP).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TAKIZAWA, Toshiaki** [JP/JP]; 2-17-43, Noguchicho, Higashimurayama, Tokyo 189-0022 (JP). **TAMIYA, Masaki** [JP/JP]; 2-17-43, Noguchicho, Higashimurayama, Tokyo 189-0022 (JP). **SAITO, Naohiro** [JP/JP]; 1-6-42 Meguritacho, Higashimurayama, Tokyo 189-0025 (JP). **KANKE, Toru** [JP/JP]; 2-17-43 Noguchicho, Higashimurayama, Tokyo 189-0022 (JP). **MATSUMOTO, Jiro** [JP/JP]; 4-21-25 Sayamadai, Sayama, Saitama 350-1304 (JP). **WADA, Yasushi** [JP/JP]; 6-29-31 Sunagawacho, Tachikawa, Tokyo 190-0031 (JP). **KAWAGOE, Junichi** [JP/JP]; 785-1-1206, Minamiotsuka, Kawagoe, Saitama 350-1162 (JP). **FERRELL, William, R.** [GB/GB]; 91 Balshagray Avenue, Glasgow G11 7EQ (GB). **LOCKHART, John, C.** [US/GB]; 9 Ryat Green, Newton Mearns, Glasgow G77 6QP (GB).

(74) Agent: **CRESSWELL, Thomas, Anthony**; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5JJ (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/049723 A2

(54) Title: PHARMACEUTICAL COMPOSITION FOR DELAYED HYPERSENSITIVITY

(57) Abstract: The present invention provides a pharmaceutical composition by a novel action mechanism without serious side effects for delayed hypersensitivity and a screening method of the same. The invention also provides a novel assay method of inhibitors/suppressive agents of PAR-2. The invention relates to a pharmaceutical composition for delayed hypersensitivity containing one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression and a pharmaceutically acceptable carrier, and to a method for screening active ingredients for pharmaceutical composition for delayed hypersensitivity by contacting a subject substance with cells expressing PAR-2 and by determining expression or activity of PAR-2. The invention also relates to a method for detecting or quantifying actions of the subject substance for PAR-2 using cells expressing PAR-2 in a culture containing inositol.

PHARMACEUTICAL COMPOSITION FOR DELAYED HYPERSENSITIVITY

Field of the Invention

The present invention relates to a pharmaceutical composition for delayed hypersensitivity of which active ingredient is an inhibitor of PAR(protease-activated receptor)-2 and/or a suppressive agent for expression of PAR-2 gene. The present invention also relates to a method for screening active ingredients for a pharmaceutical composition for delayed hypersensitivity consisting of screening subject materials for inhibition of PAR-2 or suppression of PAR-2 gene expression. Further, the present invention relates to an assay method for PAR-2 based on production of inositol phosphate as an indicator.

Background of the Invention

Allergy (hypersensitivity) is a state of a living body where immune reaction is induced in excessive or inappropriate manners and, in some cases, tissue is damaged.

Allergic responses (hypersensitivity) are induced in the second contact with an identical antigen, and classified into two types, i.e., an immediate hypersensitivity in which humoral immunity is involved, and a delayed hypersensitivity in which cellular immunity is involved. Further, Coombs and Gell divided them into I to IV types according to the differences

of occurrence mechanisms and symptoms. In the type I of hypersensitivity, immunoglobulin E antibody which binds to mast cells in tissues and basophiles in blood reacts with its specific antigen and then degranulation is induced to release chemical mediators such as histamine. The released chemical mediators act against various organs resulting in acute inflammation. In the type II of hypersensitivity, an antibody binds to an antigen on the surface of self or foreign cells, which are phagocytized, activate killer cells and induce cytolysis involving complements. In the type III of hypersensitivity, immune complexes in which complements bind to antigen/antibody conjugations formed in vivo by entered antigens deposit in tissues, which activate complements, and polymorphonuclear leukocytes are congregated around the deposit sites, resulting in local lesions. In the type IV hypersensitivity, i.e., delayed hypersensitivity, chemical mediators having biological activity such as cytokines are liberated and released by direct reaction between an antigen and T cells capable of reacting specifically to the antigen, resulting in assembling cells in a local tissue to induce inflammation.

The delayed hypersensitivity includes tuberculin reaction, rejection in allogenic transplantation, cellular defense reaction of infection, contact dermal hypersensitivity and the like, and these reactions are known to be suppressed

most strongly by steroid drugs. Therefore, the steroid drugs are effective for the diseases caused through delayed hypersensitivity, however, the steroid drugs are problematic in that discontinuation and regimen of medication are difficult because a serious side effect, i.e., dependency on steroids occurs when the steroid drugs are used for a long time. Also, in treatment of contact dermatitis, a contact dermal hypersensitivity with the steroid drugs, various side effects occur by alteration of skin conditions such as atrophy of skin, acne, hirsutism and the like, and the skin conditions conversely deteriorate in some cases.

There are a wide variety of chronic diseases by delayed hypersensitivity. They include those by non-infectious substances such as sarcoidosis and Crohn's disease, and those by infectious microbes such as bacteria, protozoa, fungi and the like. The diseases are believed to be caused by chronic antigen stimuli afforded by these microbes. It is believed that tissue disorders are induced by persistent stimuli although infection is often limited locally by activation of macrophages and the like. These infectious diseases include tuberculosis, lepra (Hansen's disease), schistosomiasis and the like.

On the other hand, PAR (protease-activated receptor)-2 is one type of PARs belonging to 7-transmembrane G-protein-coupled receptor family. Currently, four types of PARs

have been cloned, i.e., PAR-1, PAR-2, PAR-3 and PA-4, and belong to a receptor family which mediates actions of serine proteases such as thrombin, trypsin and the like in various cells. All of PAR-1, PAR-3 and PAR-4 have been demonstrated to have functions as receptors which relate to platelet aggregation by thrombin. However, PAR-2 is functionally distinct from the other PARs since it is activated by trypsin and tryptase but not by thrombin although it has a structure and activation mechanisms in common with other PARs.

In these PARs, the specific site at N-terminus of the amino acid sequence of the receptor is cleaved by the action of thrombin or other proteases, and activation of the receptor is caused by binding of the newly-exposed terminus to the binding site of the receptor per se. The summary of the amino acid sequences at the cut end which activate the receptors is represented by the single character code for amino acids.

PAR-1	SFLLRN-NH_2	(human)
PAR-2	SLIGKV-NH_2	(human)
	SLIGRL-NH_2	(mouse)
PAR-3	not available	
PAR-4	GYPGQV	(human)
	GYPGKF	(mouse)

PAR-1, PAR-2 and PAR-4 can be activated non-enzymatically by exogenouslyadded peptides having the amino acid sequences

of the cut ends, but PAR-3 can not be activated by such foreign peptides. In a recent study, it has been proved that murine PAR-3 is not activated by itself and is a cofactor of PAR-4 which can function only in the presence of PAR-4 (Nature, 404:609-613, 2000).

PAR-2 is known to be activated by tissue factors/VIIa factor; Xa factor; acrosin, a type of sperm proteases; trypsin-like serine protease identified from rat brain; trypsin; tryptase; and synthetic peptides having similar sequence to the ligand of PAR-2.

Several reports using trypsin, tryptase, PAR-2 activating peptides and PAR-2 knockout mice have shown physiological and pathological roles of PAR-2 in respect to inflammation and hypersensitivity (Br. J. Pharmacol., 125: 419-422,1988; Br. J. Pharmacol., 127:1083-1090, 1999; Eur. J. Pharmacol., 328:89-97, 1997; J. Pharmacol. Exp. Ther., 288:671-678, 1999; and J. Immunol., 165:6504-6510, 1999).

It has been reported that transient inflammatory responses are induced by an agonist of PAR-2 (SLIGRL-NH_2)(Br. J. Pharmacol., 125:419-422, 1998). However, mRNA for PAR-2 was not detected by a RT-PCR method in peritoneal mast cells, and degranulation by the PAR-2 agonist was not observed in mast cells (Jpn. J. Pharmacol., 82:74-77, 2000). Thus, the transient inflammatory response by the PAR-2 agonist (SLIGRL-NH_2) is believed to be

highly potential of secondary response via actions for cells/tissues other than mast cells. It has been also reported that nervous inflammation is exerted not depending on mast cells in experiments using high concentrations of the PAR-2 agonist ($SLIGRL-NH_2$)(Br. J. Pharmacol., 127:1083-1090, 1999; and Nat. Med., 6:151-158, 2000).

However, they are indirect evidences of PAR-2 regarding to immediate hypersensitivity, and nervous inflammation or reports of PAR-2 regarding to circulatory organs, and have not suggested roles of PAR-2 in delayed hypersensitivity.

There is a report that the inhibitor of tryptase is effective for treatment of dermatitis in the murine model of delayed hypersensitivity, but an involvement of PAR-2 is not suggested at all in the report (J. Med. Chem., 41:4854-4860, 1998). Tryptase is known to decompose and activate lots of ligands other than PAR-2, such as VIP (vasoactive intestinal peptide), PHM (peptide histidine methionine), CGRP (calcitonin gene related peptide), fibrinogen, gelatinase, fibronectin, IV type of collagen, MMP (matrix metalloprotease), uPA (urinary type plasminogen activator), and kininogenase (Pharmacol. Rev., 53:245-282, 2001), and which mechanism is effective for treatment of dermatitis in the murine model of delayed hypersensitivity has not been described. The present inventors showed that PAR-2 is scarcely involved in the dermatitis model called passive

cutaneous anaphylaxis in which tryptase is released from mast cells in the experiments using PAR-2 knockout mice. The relationship between suppression of PAR-2 activation and delayed hypersensitivity has not been disclosed in this report(J. Med. Chem., 41:4854-4860, 1998).

Summary of the Invention

The present invention provides a pharmaceutical composition for delayed hypersensitivity via a novel action mechanism without any serious side effects and methods for screening the same. The invention also provides a novel assay method for inhibitors of PAR-2 and/or suppressive agents of PAR-2 expression.

As the results of intensive study, the present inventors have found that PAR-2 is involved in reactions of delayed hypersensitivity, have found that an inhibitor of PAR-2 and/or a suppressive agent of PAR-2 gene expression is a pharmaceutical composition for delayed hypersensitivity with few manifestation of side effects, and have completed the invention based on these findings.

That is, the present invention relates to a pharmaceutical composition for delayed hypersensitivity containing one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and/or suppressive agents of PAR-2 gene

expression, and a pharmaceutically acceptable carrier.

The invention also relates to a method for screening active ingredients for pharmaceutical composition for delayed hypersensitivity comprising screening subject materials for inhibitory action against PAR-2 or suppressive action against PAR-2 gene expression by contacting the subject materials to cells on which PAR-2 is expressed followed by determining expression or activity of PAR-2.

Further, the invention relates to a method for detection or quantification of PAR-2 activation by incubation of PAR-2 expressing cells with a medium containing inositol followed by detection or quantification of inositol phosphates in the PAR-2 expressing cells incubated with the medium containing a subject material.

PAR-2 was cloned by Nystedt et al. in 1994 (Proc. Natl. Acad. Sci. USA, 91:9208-9212, 1994). The base sequence of PAR-2 and the amino acid sequence of its coding region are shown in the sequence number 1 in the sequence lists.

The production of PAR-2 gene knockout mice (PAR-$2^{-/-}$ mice) has been reported by Damiano in 1999 (J. Pharmacol. Exp. Ther., 288:671-678, 1999).

The present inventors have found that PAR-2 is involved in reactions of delayed hypersensitivity when analyzing functions of PAR-2 using PAR-$2^{-/-}$ mice.

First, the present inventors examined whether skin atrophy was exerted by deletion of PAR-2 gene by measuring ear pinna thicknesses of wild type and PAR-$2^{-/-}$ mice.

The average thicknesses of ear pinna in non-treated wild type mice and PAR-$2^{-/-}$ mice are 22.7±0.4($\times 10^{-2}$mm; the mean ± standard error) and 22.0±0.4($\times 10^{-2}$mm; the mean ± standard error), respectively, and no statistical difference was observed between them. No visible difference between them was observed with the naked eye, indicating that no skin atrophy was caused by the inhibition of PAR-2.

Next, the models for picryl chloride (PC) induced contact dermatitis and oxazolone (Ox) induced contact dermatitis were tested using PAR-$2^{-/-}$ mice, and then significant difference was observed between wild type and PAR-$2^{-/-}$ mice.

Fig. 1A (upper of Fig. 1) shows the results of ear pinna edema in the model for picryl chloride (PC) induced contact dermatitis, and Fig. 1B (lower of Fig. 1) shows results of ear pinna edema in the model for oxazolone (Ox) induced contact dermatitis. Each vertical axis in Figs 1A and 1B denotes the difference of ear pinna thickness before and after the induction when 1% PC-olive oil solution (Fig. 1A) or 0.5% Ox-acetone solution (Fig. 1B) was applied on the both sides of ear pinna to induce edema in the sensitized mice and ear pinna thickness was measured after 24 hours. The left is from the wild type mice

and the right is from the PAR-2$^{-/-}$ mice in Fig. 1A and 1B.

No effect of the solvent (olive oil or acetone) application was observed in these experiments.

In the wild type mice, incidences of edema of which peak was at 24 hours after challenge of PC or Ox were observed, and edema and redness of ear pinnas were macroscopically observed. On the contrary in the PAR-2$^{-/-}$ mice, edema exerted by PC challenge was obviously suppressed (Fig. 1A), and edema exerted by Ox was completely suppressed at 24 hour after the challenge when the peak of ear pinna edema was observed in the wild type mice.

Further, histological examination of these edema was carried out.

The thin slice specimens of both ear pinnas of the mice used for PC- or Ox-induced contact dermatitis model experiments were stained with hematoxylin-eosin to histologically observe the effects of PAR-2 deletion on PC- or Ox-induced contact dermatitis.

Fig. 2 is color photographs instead of drawings which show histopathological pictures at 24 hours after PC or Ox application to the wild type mice (the left side of Fig. 2, A, C and E) and the PAR-2$^{-/-}$ mice (the right side of Fig. 2, B, D and F). The upper panels of Fig.2 show the cases of solvent (olive oil) treatment, and the left (Fig. 2A) is from the wild type and the right (Fig. 2B) is from the PAR-2$^{-/-}$ mouse. The middle panels

of Fig.2 (Fig. 2C and 2D) show the cases of PC treatment, and the left (Fig. 2C) is from the wild type and the right (Fig. 2D) is from the PAR-2$^{-/-}$ mouse. The lower panels of Fig.2 (Fig. 2E and 2F) show the cases of Ox treatment, and the left (Fig. 2E) is from the wild type and the right (Fig. 2F) is from the PAR-2$^{-/-}$ mouse.

Edema and infiltration by inflammatory cells such as neutrophils, macrophages, lymphocytes, eosinophils and the like were observed by application of PC or Ox on ear pinnas of the wild type mice (Fig. 2C and 2E). These edema and ininfiltration by inflammatory cells such as neutrophils, macrophages, lymphocytes, eosinophils and the like were remarkably suppressed in the PAR-2$^{-/-}$ mice (Fig. 2D and 2F). In the histological observation, the incidences of edema were found to be obviously suppressed in the PAR-2$^{-/-}$ mice in these experiments.

Further, the tests were conducted in the model for passive cutaneous anaphylaxis(PCA). Antiserium against ovalbumin (OA) was subcutaneously administered in both ear pinnas of mice. After 48 hours, the solution in which Evans' Blue solution and OA solution are blended equivalently was injected intravenously. After 30 min, the ear pinnas were cut and the dye was extracted to quantify an dye leakage quantity by measuring an absorbance (λ=620 nm) by a spectrophotometer.

The results are shown in Fig. 3. The vertical axis of Fig.

3 shows the dye leakage quantity (μg), and the left is from the wild type mice and the right is from the PAR-$2^{-/-}$ mice. As shown in Fig. 3, PCA reaction was induced both in the wild type and PAR-$2^{-/-}$ mice, and no obvious effect of PAR-2 deletion was observed.

Contact dermatitis by delayed hypersensitivity has been known to be exerted by PC(2,4,6-trinitro-chlorobenzene), Ox(4-ethoxymethylene-2-phenyl-2-oxazoline-5-one) and other hapten antigens, and it has been known that inflammatory cells such as neutrophils, various cytokines and mediators are involved in its reactions. Among them, tryptase which activates PAR-2 is one of serine proteases having a wide variety of physiological activities, and is reported to indirectly activate infiltration by neutrophils and eosinophils via PAR-2 expressed on the surfaces of vascular endothelial cells. Such events have been confirmed by the evidences that PAR-2 selectively activating peptides facilitate IL-8 release from keratinocytes and binding of NFκB to DNA and further induce rolling and adhesion of neutrophils on vascular endothelial cells. However, the role of PAR-2 delayed hypersensitivity still remain unclear.

In the present results, it is believed that absence of inflammatory response via PAR-2 contributes to remarkable suppression of contact dermatitis in the PAR-$2^{-/-}$ mice.

It is also widely known that tryptase is released from

mast cells in anaphylaxis and facilitates various inflammatory responses, but roles of PAR-2 in anaphylaxis are still unclear. In the present experiments using the typical cutaneous anaphylaxis model, the role of PAR-2 was examined, but no significant difference in PCA was observed between the wild type and PAR-2$^{-/-}$ mice, indicating that involvement of PAR-2 in cutaneous anaphylaxis is insignificant.

From these results, it is shown that PAR-2 exhibits almost similar reactions as the wild type inPCA, and that PAR-2 is scarcely involved in the dermatitis models in which tryptase is released from mast cells. However, it has been shown that PAR-2 exhibited significantly different reactions from the wild type in the delayed hypersensitivity, and that deletion of PAR-2 exhibits evident suppressive actions. That is, the present invention provides a novel means to suppress delayed hypersensitivity by different mechanisms from suppression of tryptase release from mast cells, wherein delayed hypersensitivity can be suppressed by inhibiting PAR-2 activity or suppressing PAR-2 gene expression.

Therefore, the present invention first demonstrates that delayed hypersensitivity can be suppressed by substances which inhibit PAR-2 (antagonist) and can suppress expression of PAR-2 gene.

The invention also provides a novel method for measuring

PAR-2 activity. PAR-2 belongs to G-protein-coupled receptor (GPCR) family, and produces inositol phosphates as a second messenger upon activation of the receptor. Based on this mechanism, the effects of subject substances in activation of PAR-2 can be determined quantitatively by quantifying produced inositol phosphates in PAR2-expressing cells by an ion chromatography or the like.

Activation of PAR-2 by trypsin or PAR-2 activating peptide, SLIGKV, could be determined by this method. Trypsin-mediated activation was inhibited by a trypsin inhibitor derived from soybeans, while SLIGKV-mediated activation was notaffected. This manifests that inhibition of enzymes such as trypsin or tryptase is essentially different from the direct inhibition of the receptor.

The method for measuring PAR-2 activity of the invention can be widely applied, such as to screening of activation agents or inhibitors of PAR-2.

Brief Description of the Drawings

Fig. 1 shows the results of picryl chloride(PC)- or oxazolone(Ox)-induced delayed allergic response experiments in wild type and PAR-2$^{-/-}$ mice. Fig. 1A shows the results of PC-induced contact dermatitis model, and Fig. 1B shows the results of Ox-induced contact dermatitis model. The left and right sides

denote the cases of the wild type mice and PAR-$2^{-/-}$ mice, respectively in each Figure. The vertical axis in Fig. 1A and 1B each denotes the difference of ear pinna thickness before and after the induction (x 10^{-2} mm). Each numerical value represents the mean ± standard error. The symbol ** means that significant difference ($P<0.01$) exists.

Fig. 2 is color photographs instead of drawings, showing histological pathological features of specimens with hematoxylin-eosin staining of PC- or Ox-induced delayed allergic reactions in the wild type and PAR-$2^{-/-}$ mice. The wild type mice (Figs. 2A, 2C and 2E) and PAR-$2^{-/-}$ mice (Figs. 2B, 2D and 2F) were treated with solvent alone (olive oil; Figs. 2A and 2B), PC (Figs. 2C and 2D), or Ox (Figs. 2E and 2F). The bar denotes 100 μm in length.

Fig. 3 shows the results of passive cutaneous anaphylaxis reaction tests in the wild type and PAR-$2^{-/-}$ mice. The vertical axis in Fig. 3 denotes the leakage quantities of the dye (μg). The left and right are the cases of wild type and PAR-$2^{-/-}$ mice, respectively. The columns and bars represent the mean ± standard error in each group.

Description of the Preferred Embodiments

The present invention provides a pharmaceutical composition for delayed hypersensitivity containing one or two

or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression, and a pharmaceutically acceptable carrier. The invention also provides a method for preventing/treating delayed hypersensitivity comprising administering a medicine containing an effective amount of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression, to a patient with delayed hypersensitivity. Further, the invention provides use of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression for producing a pharmaceutical composition for delayed hypersensitivity.

The inhibitor of PAR-2 or suppressive agent of PAR-2 gene expression of the invention is one which can inhibit PAR-2 activity or suppress expression of PAR-2 gene by a method for screening using PAR-2 expressing cells.

The inhibitor of PAR-2 or suppressive agent of PAR-2 gene expression of the invention has a suppressive action against delayed hypersensitivity, and is useful as a pharmaceutical composition for contact dermatitis, graft rejection, graft versus host disease , tuberculin reaction, granulation, tuberculosis, lepra, sarcoidosis, Crohn's disease, chronic ulcerative colitis, schistosomiasis, autoimmune diseases (such

as rheumatoid arthritis, systemic lupus erythematosus, chronic ulcerative colitis, myasthenia gravis, insulin dependent diabetes mellitus, Hashimoto's thyroiditis, scleroderma, pernicious anemia), atopic dermatitis, asthma, chronic obstructive pulmonary disease, rhinitis, allergic conjunctivitis, food allergy, nephritis, and diseases with inflammatory infiltration by one or more types of neutrophils, macrophages, lymphocytes and eosinophils.

The inhibitor of PAR-2 of the invention can be used alone or can be used in combination with the other pharmaceutically acceptable resolvents, excipients, binders, diluents and the like to be formulated into tablets, capsules, granules, powder, lotion, ointment, injection, suppository and the like. These preparations can be produced by the methods known in the art. For example, the preparations for oral administration can be produced by formulating in appropriate combination with resolvents such as gum tragacanth, gum arabic, sucrose ester, lecithin, olive oil, soybean oil, PEG400 and the like; excipients such as starch, mannitol, lactose and the like; binders such as sodium carboxymethyl cellulose, hydroxypropyl cellulose and the like; disintegrants such as crystal cellulose, calcium carboxymethyl cellulose and the like, lubricants such as talc, magnesium stearate and the like; fluidity improvers such as silicic anhydride light and the like.

The inhibitor of PAR-2 of the invention is administered orally or parenterally.

The dosage of the inhibitor of PAR-2 of the invention varies depending on body weight, age, sex, condition and the like of the patient, is usually from 0.01 to 1000 mg per day in the adult, and preferably it is preferred to administer from 0.1 to 100 mg by dividing into 1 to 3 times in a day.

The invention also provides a method for screening active ingredients for pharmaceutical composition for delayed hypersensitivity comprising screening subject substances for inhibitory action against PAR-2 or suppressive action against PAR-2 gene expression by contacting the subject substances to cells on which PAR-2 is expressed followed by determining expression or activity of PAR-2.

The cells expressing PAR-2 used for the screening method of the invention include, but not are limited to, for example, NCTC2544 cells, NEK293 cells, and the like, however, any cells which express PAR-2 of animals such as mouse, rat, human and the like can be used. For example, the screening can be performed by contacting the subject substance such as a candidate compound and the like with these cells expressing PAR-2 in a medium where the cells can be cultured and by measuring activity of PAR-2. The methods for measuring activity of PAR-2 is not specifically limited, but preferred is the measuring method by phosphorylation

of inositol described below.

The invention provides a method for detecting or quantifying actions against PAR-2 activation by culturing PAR-2 expressing cells in the medium containing inositol followed by detecting or quantifying a quantity of inositol phosphates by the stimulation with PAR-2 in the medium to which a subject substance is added.

Inositol used for the method of the invention may be any of those which are phosphorylated by activation of PAR-2, but usually myo-inositol is preferable.

Also the method for measuring phosphorylation of inositol is not specifically limited, but the method of Oldham et al. reported to be simple and sensitive is preferable (Oldham, K. G., Polyphosphoinositide turnover. In Receptor-effector coupling- A practical approach, ed., Hulme, E.C., pp.99-116, Oxford University Press, 1990). In this method, ^{3}H-myo-inositol is used in the presence of lithium salt which is an inhibitor of inositol monophosphatase. The produced phosphorylated inositol is extracted with an organic solvent or TCA solution, and then is separated from free inositol using an ion exchanging chromatography.

In this way, the method employing inositol labeled with a radioisotope is relatively simple and preferred. The radioisotopes may be any of hydrogen, oxygen and carbon atoms

of inositol, and for example, the hydrogen atoms can be labeled with radioactive ^{3}H. PAR-2 activity can be measured by detection or quantification phosphorylated inositol using such labeled inositol. This method of the invention can screen whether the subject substance has an inhibitory action against PAR-2 or a suppressive action against PAR-2 gene expression or not.

Examples

The invention is more specifically described by the following examples, but the technical scope of the invention is not limited to these examples.

The mice used in the following experiments were PAR-$2^{-/-}$ and its wild type male mice derived from a hybrid strain of C57BL/6 and 129/Ola bred in our laboratory, of ages from 6 to 9 weeks, were bred at 23±3°C, and were given feeds and water ad libitum.

Example 1

Examination on skin atrophy

The thickness of ear pinnas was measured in non-treated wild type and PAR-$2^{-/-}$ mice. The average values of each 10 mice are:

Wild type mice 22.7 ± 0.4 ($\times 10^{-2}$ mm; the mean ± standard error)

PAR-$2^{-/-}$ mice 22.0 ± 0.4 ($\times 10^{-2}$ mm; the mean ±

standard error)

and no statistical difference was observed between the two groups. Also no difference was macroscopcally observed, indicating that inhibition of PAR-2 did not cause skin atrophy.

Example 2

Picryl chloride (PC) (2,4,6-trinitro-chlorobenzene)-induced contact dermatitis model

After shaving hairs, each 10 mice of PAR-$2^{-/-}$ and wild type groups were sensitized by applying 100 μl of 7% PC-ethanol solution on the abdominal part. After 6 days, the thickness of both ear pinnas was measured using a dial thickness gauge (Peacock G-1A, OZAKI MFG. Co. Ltd.). Then, induction was performed by applying 20 μl of 1% PC-olive oil or solvent alone on the both sides of the ear pinnas, and then the thickness of ear pinnas was measured after 6, 24, and 48 hours. The difference of ear pinna thickness before and after the induction was calculated to be an indicator of edema. The results are shown in Table 1 described below.

The result after 24 hours is represented as a graph in Fig. 1A. By applying PC on the ear pinnas of the wild type mice, incidences of edema were observed, of which peak was at 24 hours after the induction. Edema and rubefaction of the ear pinnas were also observed with the naked eye. These incidences of edema

were obviously inhibited in the PAR-2$^{-/-}$ mice(Fig. 1A). No effect of application with solvent (olive oil) was found.

Example 3

Oxazolone (Ox)

(4-ethoxymethylene-2-phenyl-2-oxazoline-5-one)-induced

contact dermatitis model

After removing hairs, each 5 mice of PAR-2$^{-/-}$ and wild type groups were sensitized by applying with 100 μl of 0.5% Ox-ethanol solution on the abdominal part . After 5 days, the thickness of the ear pinnas before the induction was measured using the dial thickness gauge. Then, the induction was performed by applying with 20 μl of 0.5% Ox-acetone solution or solvent alone, and the thickness of the ear pinnas was measured after 6, 24, and 48 hours. The difference of ear pinna thickness before and after the induction was calculated to be an indicator of edema. The results are shown in Table 1 in conjunction with the above results.

Table 1

Genotype	n	Hours after the challenge		
		6 h	24 h	48 h
PC-induced contact dermatitis				
Wild type	10	1.1±0.3	9.2±1.8	7.3±1.3
PAR-$2^{-/-}$	10	0.4±0.3	2.3±1.2**	3.5±1.3
Ox-induced contact dermatitis				
Wild type	5	1.0±0.6	11.4±2.0	9.3±0.7
PAR-$2^{-/-}$	5	-0.1±0.4	-0.9±0.6**	4.1±1.8*

The numerical values denote the mean ± standard error of the ear pinna edema. ($\times 10^{-2}$ mm)

Each numerical value in Table 1 represents the mean value ± standard error of the ear pinna edema. The symbols * ($P<0.05$) and ** ($P<0.01$) denote the significant difference.

The result after 24 hours is shown in Fig. 1B as a graph. As shown in Fig. 1B and as is the case with PC, incidences of edema were observed by applying Ox on the ear pinnas of the wild type mice. The edema reaction was completely inhibited in the PAR-$2^{-/-}$ mice. No effect of application of solvent (acetone) was found.

Example 4

Histological examination

The ear pinnas were exsected from anesthetized mice used in the examinations for PC- and Ox-induced contact dermatitis models, fixed with neutral buffer formalin and embedded with paraffin to make thin sections, which were stained with hematoxylin-eosin. The effects of PAR gene deletion were histologically observed on PC- or Ox-induced contact dermatitis.

The histological and pathological features of 24 hours after the challenge with PC or Ox in the wild type and PAR-$2^{-/-}$ mice are shown in Fig. 2 as color photographs instead of the drawings. Edema and infiltration by inflammatory cells such as neutrophils, macrophages, lymphocytes, eosinophils and the like were observed by applying PC or Ox on the ear pinnas of the wild type mice (Figs. 2C and 2E). Such edema and infiltration by inflammatory cells such as neutrophils, macrophages, lymphocytes, eosinophils and the like were remarkably suppressed in the PAR-$2^{-/-}$ mice (Figs. 2D and 2F).

Example 5

Passive cutaneous anaphylaxis (PCA) model

After anesthetizing mice with ether, anti-OA anti serum (derived from BALB/c mice, PCA Titer 16 folds) diluted with saline was subcutaneously injected at 5 µl/site in both ear pinnas using a microsyringe. The solution in which 1% Evans' blue solution and 0.2% OA solution were blended equivalently was intravenously

administered at a volume of 10 ml/kg after 48 hours. The ear pinna was cut off after 30 min, to which 0.25 ml of 1N KOH was added and incubated at 37°C overnight. Then, 0.375 mL of 6N phosphoric acid and 1.125 mL of acetone were further added, and centrifuged (at 3,000 rpm for 10 min) to extract the dye. The leakage quantity of the dye was quantified by determining absorbance (λ=620 nm) using a spectrometer (UV-2200, Shimadzu Corporation).

The results are shown in Fig. 3 as a graph. The PCA reaction was exerted in the wild type and PAR-$2^{-/-}$ mice, and the levels of their reactivity were compared. As shown in Fig. 3, PCA reaction was exerted both in the wild type and PAR-$2^{-/-}$ mice, and a clear effect of PAR-2 gene deletion was not found.

Example 6

The assay method of PAR-2 using inositol phosphates as an indicator

NCTC2544 cells with stably high expression of PAR-2 were cultured in the serum-free medium containing 2 mCi/ml of [^{3}H]-myo-inositol for 18 hours. Lithium chloride (final 5 mM) was added 30 min before the stimulation, and subsequently cells were stimulated with trypsin (1 to 30 nM) or the PAR-2 agonist peptide, SLIGKV (10 to 300 μM). After 45 min, the lipid component was extracted with methanol and separated using an anion

exchanging resin (AG1-X, formate form). Then the yield of inositol phosphates was determined in a scintillation counter. The subject substance was added 15 min before the stimulation.

From the results of the above studies, it is shown that the inhibitor of PAR-2 and the suppressive agent of PAR-2 gene expression are useful as the suppressive agent for delayed hypersensitivity without any side effect. The present invention provides the novel pharmaceutical composition with few side effects for delayed hypersensitivity by suppressing causative action of delayed hypersensitivity which is elucidated by the invention. In particular, it is useful as the pharmaceutical composition for contact dermatitis, graft rejection, graft versus host disease, tuberculin reaction, granulation, tuberculosis, lepra, sarcoidosis, Crohn's disease, chronic ulcerative colitis, schistosomiasis, autoimmune diseases (such as rheumatoid arthritis, subchronic rheumatoid arthritis, juvenile subchronic rheumatoid arthritis, systemic lupus erythematosus, chronic ulcerative colitis, myasthenia gravis, insulin dependent diabetes mellitus, Hashimoto's thyroiditis, scleroderma, pernicious anemia), psoriatic arthritis, atopic dermatitis, asthma, chronic obstructive pulmonary disease, rhinitis, allergic conjunctivitis, food allergy, nephritis and diseases with inflammatory cell infiltration by one or more types of neutrophils, macrophages, lymphocytes and eosinophils, all of which are particularly caused by the delayed hypersensitivity reaction. Further, it does not cause skin atrophy which is one of side effects of topical steroid drugs currently and widely used for clinical treatment, resulting in a lower potential for manifestation of side effects.

The invention also provides the screening method for the pharmaceutical composition of delayed hypersensitivity using cells expressing PAR-2, by demonstrating that PAR-2 is involved in delayed hypersensitivity reaction. The screening method of the invention enables to simply find the novel pharmaceutical composition of delayed hypersensitivity with few side effects. Additionally, the invention provides the measuring method of PAR-2 activity. The measuring method of the invention can determine PAR-2 activity simply and accurately, and enables to detect or quantify the action of the subject substance for PAR-2.

Example 7

Chronic joint inflammation is attenuated in PAR-2 deficient mice

Intra- and peri-articular administration of Freunds complete adjuvant (FCA) resulted in a substantial increase in knee joint diameter in wild type mice which was sustained over a four week period. However, this chronic inflammatory response was markedly inhibited in PAR-2 -/-mice (fig. 4), differing significantly from wild type mice ($P<0.001$; 2-way ANOVA). All animals showed an initial inflammatory response 24hrs after injection of FCA, but the chronic inflammatory phase evident in the WT animals was notably absent in PAR-2/-mice.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for delayed hypersensitivity containing one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition for delayed hypersensitivity according to claim 1, wherein the pharmaceutical composition for delayed hypersensitivity is a suppressive agent for contact skin hypersensitive reaction and/or allogenic graft rejection.

3. The pharmaceutical composition for delayed hypersensitivity according to claim 1 or 2, wherein the pharmaceutical composition for delayed hypersensitivity is a pharmaceutical composition for contact dermatitis, graft rejection, graft versus host disease, tuberculin reaction, granulation, tuberculosis, lepra, sarcoidosis, Crohn's disease, chronic ulcerative colitis, schistosomiasis, autoimmune diseases (such as rheumatoid arthritis, subchronic rheumatoid arthritis, juvenile subchronic rheumatoid arthritis, systemic lupus erythematosus, chronic ulcerative colitis, myasthenia gravis, insulin dependent diabetes mellitus, Hashimoto's thyroiditis, scleroderma, pernicious anemia), psoriatic arthritis, atopic dermatitis, asthma, chronic obstructive pulmonary disease, rhinitis, allergic conjunctivitis, food allergy, nephritis, and diseases with inflammatory infiltration by one or more types of neutrophils, macrophages, lymphocytes and eosinophils.

4. One or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression for use in a method of preventing or treating delayed hypersensitivity.

5. Use of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression in the manufacture of a medicament for preventing or treating delayed hypersensitivity.

6. Use of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression in the manufacture of a medicament for suppressing contact skin hypersensitive reaction and/or allogenic graft rejection.

7. Use of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression in the manufacture of a medicament for preventing or treating a condition as defined in claim 3.

8. Method of preventing or treating delayed hypersensitivity comprising administering to an individual in need thereof a non-toxic effective amount of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression.

9. Method of preventing or treating a condition as defined in claim 2 or 3 comprising administering to an individual in need thereof a non-toxic effective amount of one or two or more active ingredients selected from the group consisting of inhibitors of PAR-2 and suppressive agents of PAR-2 gene expression.

10. A screening method comprising screening a subject substance for an inhibitory action against PAR-2 or a suppressive action against PAR-2 gene expression by contacting the subject substance with cells expressing PAR-2 and by determining expression or activity of PAR-2.

11. The method according to claim 10, wherein the type of cells expressing PAR-2 is NCTC2544 cell.

12. The method according to claim 10 or 11, wherein the method for screening for an inhibitory action against PAR-2 or a suppressive action against PAR-2 gene expression is achieved by a PAR-2 assay method utilizing production of inositol phosphates as an indicator.

13. A detecting or quantifying method of PAR-2 activation by detecting or quantifying a quantity of inositol phosphates by PAR-2 expressing cells in culture to which the subject substance is added following to a culture of the PAR-2 expressing cells in a medium containing inositol.

14. The method according to claim 13, wherein a type of cells expressing PAR-2 is NCTC2544 cell.

15. A substance which has inhibitory action against PAR-2 or a suppressive action against PAR-2 gene expression identified by the method of any one of claims 10 to 12.

16. Method according to any one of claims 10 to 12 which further comprises formulating the substance thus identified which has inhibitory action against PAR-2 or a suppressive action against PAR-2 gene expression into a composition for preventing or treating a condition as defined in any one of claims 1 to 3.

17. Use of a substance identified in the method of any one of claims 10 to 12 as having inhibitory action against PAR-2 or suppressive action against PAR-2 gene expression in the manufacture of a medicament for preventing or treating a condition as defined in any one of claims 1 to 3.

18. Method according to any one of claims 10 to 12 which further comprises administering a non-toxic effective amount of the identified substance to an individual in need thereof for preventing or treating a condition as defined in any one of claims 1 to 3.

19. Use of a cell expressing PAR-2 to screen for a substance capable of preventing or treating a condition as defined in any one of claims 1 to 3.

Figure 1

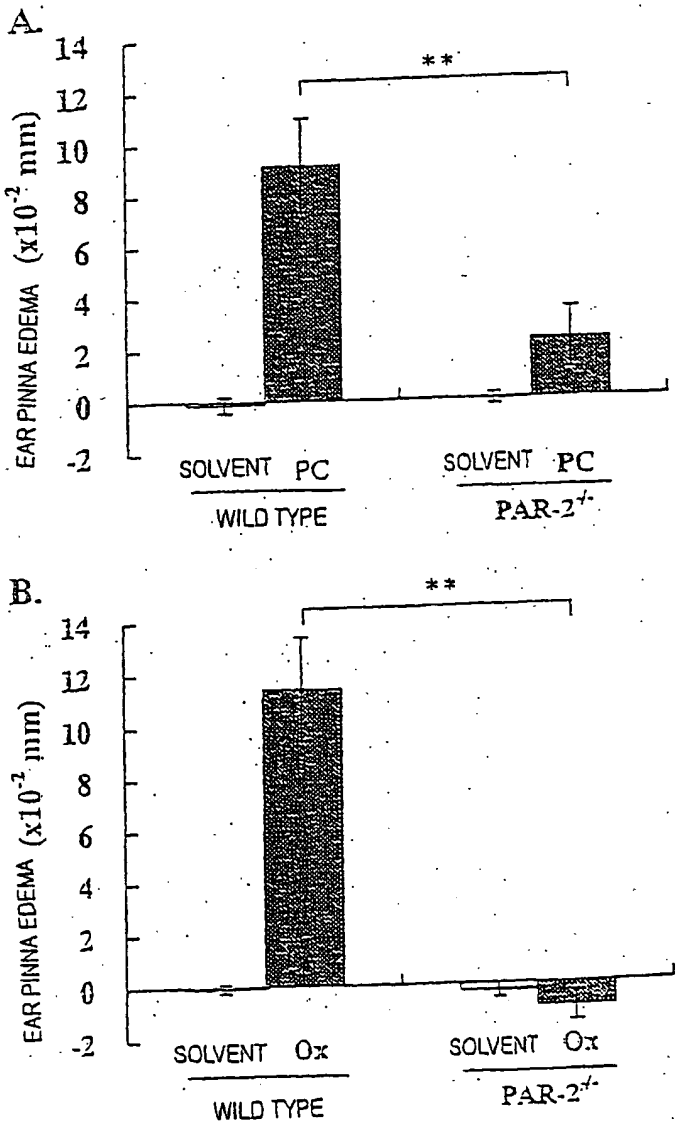

A.
EAR PINNA EDEMA (x10^{-2} mm)
14
12
10
8
6
4
2
0
-2
**
SOLVENT PC
WILD TYPE
SOLVENT PC
PAR-2$^{-/-}$
B.
EAR PINNA EDEMA (x10^{-2} mm)
14
12
10
8
6
4
2
0
-2
**
SOLVENT Ox
WILD TYPE
SOLVENT Ox
PAR-2$^{-/-}$

Figure 2

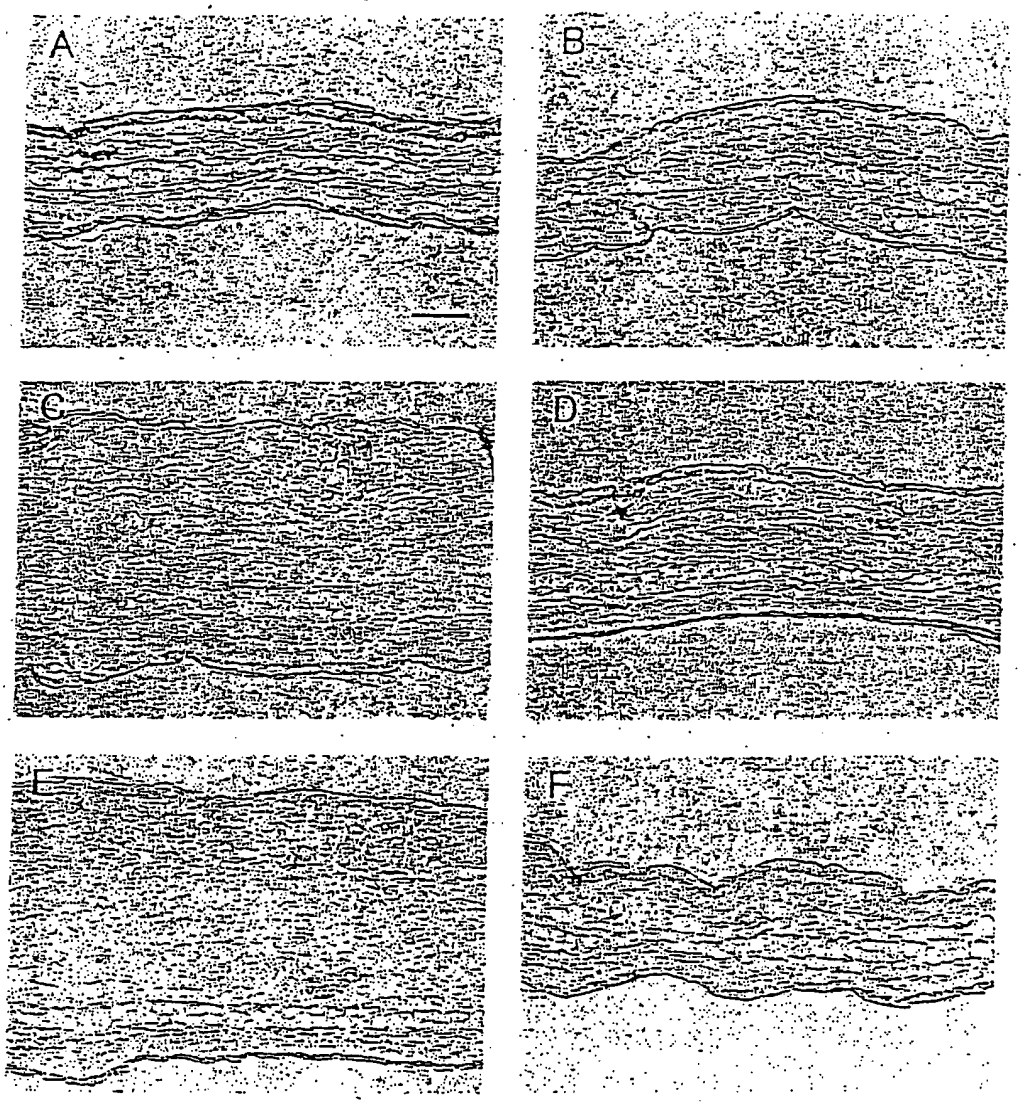


Figure 3

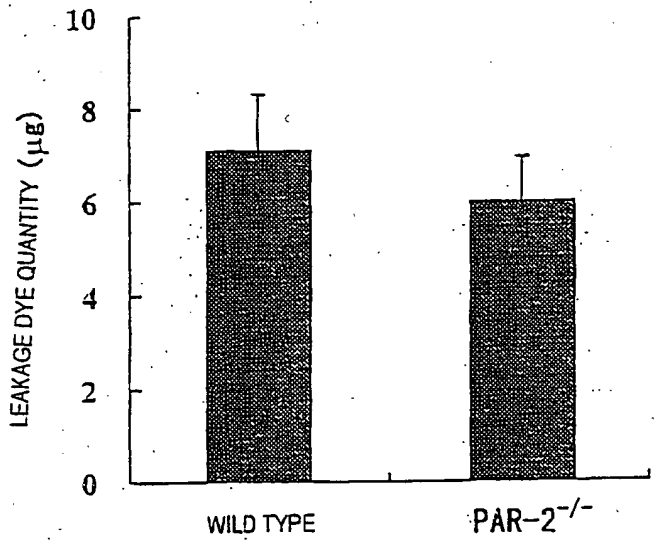
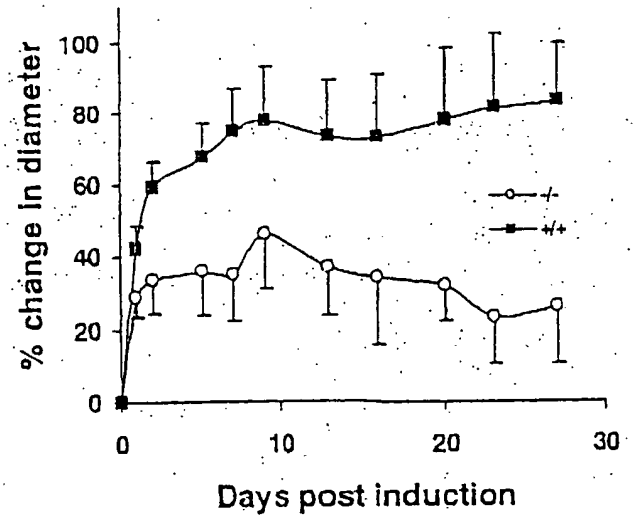


Figure 4



Intra-articular and peri-articular injection of Freunds complete adjuvant elicits a sustained increase in knee joint diameter in wild-type mice (■), the chronic phase of which is substantially attenuated in PAR-2 deficient mice (○). Means ± SEM; n = 4.

SEQUENCE LISTING

<110> Kowa Company Ltd.

<120> Pharmaceutical Composition for Delayed Hypersensitivity

<130> PA508321

<160> 2

<170> PatentIn Ver. 2.1

<210> 1
<211> 2713
<212> DNA
<213> house mouse

<220>
<221> 5'UTR
<222> (1)..(72)

<220>
<221> CDS
<222> (73)..(1272)

<220>
<221> intron
<222> (163)..(164)

<220>
<221> 3'UTR
<222> (1273)..(2713)

<220>
<221> polyA_signal

<222> (2219)..(2224)

<220>
<221> polyA_signal
<222> (2693)..(2698)

<400> 1
ccctgtgctc agagtagggc tccgagtttc gaaccactgg tggcggattg cccgcccgcc 60

ccacgtccgg gg atg cga agt ctc agc ctg gcg tgg ctg ctg gga ggt atc 111
Met Arg Ser Leu Ser Leu Ala Trp Leu Leu Gly Gly Ile
1 5 10

acc ctt ctg gcg gcc tcg gtc tcc tgc agc cgg acc gag aac ctt gca 159
Thr Leu Leu Ala Ala Ser Val Ser Cys Ser Arg Thr Glu Asn Leu Ala
15 20 25

ccg gga cgc aac aac agt aaa gga aga agt ctt att ggc aga tta gaa 207
Pro Gly Arg Asn Asn Ser Lys Gly Arg Ser Leu Ile Gly Arg Leu Glu
30 35 40 45

acc cag cct cca atc act ggg aaa ggg gtt ccg gta gaa cca ggc ttt 255
Thr Gln Pro Pro Ile Thr Gly Lys Gly Val Pro Val Glu Pro Gly Phe
50 55 60

tcc atc gat gag ttc tct gcg tcc atc ctc acc ggg aag ctg acc acg 303
Ser Ile Asp Glu Phe Ser Ala Ser Ile Leu Thr Gly Lys Leu Thr Thr
65 70 75

gtc ttt ctt ccg gtc gtc tac att att gtg ttt gtg att ggt ttg ccc 351
Val Phe Leu Pro Val Val Tyr Ile Ile Val Phe Val Ile Gly Leu Pro
80 85 90

agt aat ggc atg gcc ctc tgg atc ttc ctt ttc cga acg aag aag aaa 399
Ser Asn Gly Met Ala Leu Trp Ile Phe Leu Phe Arg Thr Lys Lys Lys
95 100 105

```
cac ccc gcc gtg att tac atg gcc aac ctg gcc ttg gcc gac ctc ctc   447
His Pro Ala Val Ile Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu
110                 115                 120                 125

tct gtc atc tgg ttc ccc ctg aag atc tcc tac cac cta cat ggc aac   495
Ser Val Ile Trp Phe Pro Leu Lys Ile Ser Tyr His Leu His Gly Asn
                130                 135                 140

aac tgg gtc tac ggg gag gcc ctg tgc aag gtg ctc att ggc ttt ttc   543
Asn Trp Val Tyr Gly Glu Ala Leu Cys Lys Val Leu Ile Gly Phe Phe
            145                 150                 155

tat ggt aac atg tat tgc tcc atc ctc ttc atg acc tgc ctc agc gtg   591
Tyr Gly Asn Met Tyr Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val
        160                 165                 170

cag agg tac tgg gtg atc gtg aac ccc atg gga cac ccc agg aag aag   639
Gln Arg Tyr Trp Val Ile Val Asn Pro Met Gly His Pro Arg Lys Lys
    175                 180                 185

gca aac atc gcc gtt ggc gtc tcc ttg gca atc tgg ctc ctg att ttt   687
Ala Asn Ile Ala Val Gly Val Ser Leu Ala Ile Trp Leu Leu Ile Phe
190                 195                 200                 205

ctg gtc acc atc cct ttg tat gtc atg aag cag acc atc tac att cca   735
Leu Val Thr Ile Pro Leu Tyr Val Met Lys Gln Thr Ile Tyr Ile Pro
                210                 215                 220

gca ttg aac atc acc acc tgt cac gat gtg ctg cct gag gag gta ttg   783
Ala Leu Asn Ile Thr Thr Cys His Asp Val Leu Pro Glu Glu Val Leu
            225                 230                 235

gtg ggg gac atg ttc aat tac ttc ctc tca ctg gcc att gga gtc ttc   831
Val Gly Asp Met Phe Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe
        240                 245                 250
```

```
ctg ttc ccg gcc ctc ctt act gca tct gcc tac gtg ctc atg atc aag  879
Leu Phe Pro Ala Leu Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Lys
    255                 260                 265

acg ctc cgc tct tct gct atg gat gaa cac tca gag aag aaa agg cag  927
Thr Leu Arg Ser Ser Ala Met Asp Glu His Ser Glu Lys Lys Arg Gln
270                 275                 280                 285

agg gct atc cga ctc atc atc acc gtg ctg gcc atg tac ttc atc tgc  975
Arg Ala Ile Arg Leu Ile Ile Thr Val Leu Ala Met Tyr Phe Ile Cys
                290                 295                 300

ttt gct cct agc aac ctt ctg ctc gta gtg cat tat ttc cta atc aaa  1023
Phe Ala Pro Ser Asn Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys
            305                 310                 315

acc cag agg cag agc cac gtc tac gcc ctc tac ctt gtc gcc ctc tgc  1071
Thr Gln Arg Gln Ser His Val Tyr Ala Leu Tyr Leu Val Ala Leu Cys
        320                 325                 330

ctg tcg acc ctc aac agc tgc ata gac ccc ttt gtc tat tac ttt gtc  1119
Leu Ser Thr Leu Asn Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val
    335                 340                 345

tca aaa gat ttc agg gat cac gcc aga aac gcg ctc ctc tgc cga agt  1167
Ser Lys Asp Phe Arg Asp His Ala Arg Asn Ala Leu Leu Cys Arg Ser
350                 355                 360                 365

gtc cgc act gtg aat cgc atg caa atc tcg ctc agc tcc aac aag ttc  1215
Val Arg Thr Val Asn Arg Met Gln Ile Ser Leu Ser Ser Asn Lys Phe
                370                 375                 380

tcc agg aag tcc ggc tcc tac tct tca agc tca acc agt gtt aaa acc  1263
Ser Arg Lys Ser Gly Ser Tyr Ser Ser Ser Ser Thr Ser Val Lys Thr
            385                 390                 395
```

tcc tac tga gctgtacctg aggatgtcaa gcctgcttga tgatgatgat 1312
Ser Tyr
400

gatgatggtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgca cccgtgtgtg 1372

agtgcgtggt agggatgcac caacatgcat ggggctgtca tttcctatcc aagctgtctg 1432

tctctgcacc aatcacaagc atgcagctct ccccaggatt gccagaagcc tcctcctttg 1492

catgagaaca gtcttccact ctgatgaaaa gcatcagtat cagaaactga aacgaactga 1552

gaggagcttg ttttgtgaaa gtgaagagaa gatggagggt cagtgacttg caaaaaaaac 1612

caaccaaaca aaaactacac ctggcaagaa ggctaagact ctctgaaatg cttccttttc 1672

catctggagt tcgtctcggc cttgttcagg acctgaggcc ctggtagagc ttcagtccag 1732

ttgattgact ttacagactt gagagaggag tgaatgagga gtgaatgagg ctcctggcgg 1792

catcctaacc ggctaacagt ggccttgctg gacaatagga ttcagatggc tggagttaca 1852

ttctcacacc atttcatcag aactattggg gatcttgatc aatgtgcagg tcccttagcg 1912

tcagtaaccc tgggagctca gacacgatgg gggtgagggt gggggtgggg gtgggggtga 1972

ggctctacaa accttagtga tgactgcaga cacagaacca tggagctgag cctgcttctg 2032

cttgccaggg caccactgta atgttggcaa agaaaaacca acagcagtgt tttgagcctc 2092

ttttttggt cagtttatga tgaatttgcc tattggttta ttgggatttt cagttccttt 2152

attactttgt tgtaattttg tgtgtttatt agtcaagaaa aagaagatga ggctcttaaa 2212

aatgtaaata aaatttttgg ttttttggtt ttttaacttg ggccaactac aaatactgct 2272

taggtttttt tctaacttaa ttgttaacta catcatgtga acttaagaca ttttcatgat 2332

aaagcattac tgtagtgtca gttttccctc atcctcgatc atagtccttc ccgtgaagca 2392

gggcccttcc cctcccccc ctttgccgtt tccctcccca ccagatagtc ccctgtctgc 2452

tttaacctac cagttagtat tttataaaaa cagatcattg gaatatttat tatcagtttt 2512

gttcactgtt atcagttttg ttcactaatt tgtccaataa tggaattaac gtcttctcat 2572

ctgtttgagg aagatctgaa acaaggggcc attgcaggag tacatggctc caggcttact 2632

ttatatactg cctgtatttg tggctttaaa aaaatgacct tgttatatga atgctttata 2692

aataaataat gcatgaactt t 2713

<210> 2
<211> 399
<212> PRT
<213> house mouse

<400> 2

Met Arg Ser Leu Ser Leu Ala Trp Leu Leu Gly Gly Ile Thr Leu Leu
1 5 10 15
Ala Ala Ser Val Ser Cys Ser Arg Thr Glu Asn Leu Ala Pro Gly Arg
20 25 30
Asn Asn Ser Lys Gly Arg Ser Leu Ile Gly Arg Leu Glu Thr Gln Pro
35 40 45
Pro Ile Thr Gly Lys Gly Val Pro Val Glu Pro Gly Phe Ser Ile Asp
50 55 60
Glu Phe Ser Ala Ser Ile Leu Thr Gly Lys Leu Thr Thr Val Phe Leu
65 70 75 80
Pro Val Val Tyr Ile Ile Val Phe Val Ile Gly Leu Pro Ser Asn Gly
85 90 95

```
Met Ala Leu Trp Ile Phe Leu Phe Arg Thr Lys Lys Lys His Pro Ala
            100                 105                 110
Val Ile Tyr Met Ala Asn Leu Ala Leu Ala Asp Leu Leu Ser Val Ile
        115                 120                 125
Trp Phe Pro Leu Lys Ile Ser Tyr His Leu His Gly Asn Asn Trp Val
    130                 135                 140
Tyr Gly Glu Ala Leu Cys Lys Val Leu Ile Gly Phe Phe Tyr Gly Asn
145                 150                 155                 160

Met Tyr Cys Ser Ile Leu Phe Met Thr Cys Leu Ser Val Gln Arg Tyr
                165                 170                 175
Trp Val Ile Val Asn Pro Met Gly His Pro Arg Lys Lys Ala Asn Ile
            180                 185                 190
Ala Val Gly Val Ser Leu Ala Ile Trp Leu Leu Ile Phe Leu Val Thr
        195                 200                 205
Ile Pro Leu Tyr Val Met Lys Gln Thr Ile Tyr Ile Pro Ala Leu Asn
    210                 215                 220
Ile Thr Thr Cys His Asp Val Leu Pro Glu Glu Val Leu Val Gly Asp
225                 230                 235                 240
Met Phe Asn Tyr Phe Leu Ser Leu Ala Ile Gly Val Phe Leu Phe Pro
                245                 250                 255
Ala Leu Leu Thr Ala Ser Ala Tyr Val Leu Met Ile Lys Thr Leu Arg
            260                 265                 270
Ser Ser Ala Met Asp Glu His Ser Glu Lys Lys Arg Gln Arg Ala Ile
        275                 280                 285
Arg Leu Ile Ile Thr Val Leu Ala Met Tyr Phe Ile Cys Phe Ala Pro
    290                 295                 300
Ser Asn Leu Leu Leu Val Val His Tyr Phe Leu Ile Lys Thr Gln Arg
305                 310                 315                 320
Gln Ser His Val Tyr Ala Leu Tyr Leu Val Ala Leu Cys Leu Ser Thr
                325                 330                 335
Leu Asn Ser Cys Ile Asp Pro Phe Val Tyr Tyr Phe Val Ser Lys Asp
            340                 345                 350
Phe Arg Asp His Ala Arg Asn Ala Leu Leu Cys Arg Ser Val Arg Thr
        355                 360                 365
Val Asn Arg Met Gln Ile Ser Leu Ser Ser Asn Lys Phe Ser Arg Lys
```

370 375 380

Ser Gly Ser Tyr Ser Ser Ser Ser Thr Ser Val Lys Thr Ser Tyr

385 390 395

KS COMMENTS 09/10/05

ASSET PURCHASE AGREEMENT

AMONG

NUI SERVICE, INC.
As Seller

AGL RESOURCES, INC.
As Indirect Owner of Seller

AND

AMERICA'S WATER HEATER RENTALS, LLC
As Purchaser

Dated as of September 15, 2005

TABLE OF CONTENTS

ASSET PURCHASE AGREEMENT

This Asset Purchase Agreement (this "**Agreement**") is entered into as of September 15, 2005, by and among NUI Service, Inc., a New Jersey corporation ("**Seller**"), AGL Resources, Inc. ("**AGL**"), a Georgia corporation, and America's Water Heater Rentals, LLC, a Delaware limited liability company ("**Purchaser**"). Purchaser, AGL and Seller are referred to collectively herein as the "**Parties**."

Whereas, Seller owns and operates a business (the "**Business**") in which it leases, services and sells gas water heaters, gas ranges and gas clothes dryers (the "**Appliances**") in Florida;

Whereas, AGL is the sole, ultimate owner of Seller;

Whereas, Purchaser wishes to purchase from Seller, and Seller wishes to sell to Purchaser, certain assets related to or used in connection with the Business, all in accordance with and subject to the terms and conditions set forth in this Agreement; and

Whereas, Purchaser wishes to assume from Seller, and Seller wishes to assign to Purchaser, certain liabilities incurred by Seller in connection with or related to the Business, all in accordance with and subject to the terms and conditions set forth in this Agreement.

Now, therefore, in consideration of the premises and the mutual promises herein made, and in consideration of the representations, warranties, and covenants herein contained, the Parties agree as follows:

Section 1. *Definitions*. Capitalized terms used in this Agreement shall for all purposes of this Agreement have the respective meanings assigned to such terms in Annex I attached hereto.

Section 2. *Purchase and Sale.*

(a) *Purchase and Sale of Assets*. On and subject to the terms and conditions of this Agreement, Purchaser hereby purchases from Seller, and Seller hereby sells, transfers, conveys, and delivers (or makes available pursuant to **Section 5(b)**) to Purchaser all of the right, title and interest that Seller possesses and has the right to transfer in the following (the "**Acquired Assets**") at the Closing for the consideration specified below in **Sections 2(c)** and **(d)**:

(i) inventories of manufactured and purchased parts, raw materials, supplies and goods in process, in each case with respect to Appliances, and finished goods consisting of Appliances ready for use in the Business (the "**Inventory**") and not already subject to a lease by Seller to any Person who leases or purchases an Appliance from Seller (a "**Customer**");

(ii) all leases of Appliances to Customers, and rights thereunder;

(iii) all Appliances subject to a lease, currently in possession of Customers;

(iv) accounts, notes, and other receivables from Customers, in each case with respect to the Business; and

(v) copies of books, records, ledgers, files, documents, and lists (including Seller's Customer database), in each case with respect to the Business.

(b) *Excluded Assets.* The Acquired Assets shall not include any of the following (collectively, the "**Excluded Assets**"):

(i) the corporate charter, qualifications to conduct business as a foreign corporation, arrangements with registered agents relating to foreign qualifications, taxpayer and other identification numbers, seals, minute books, stock transfer books, blank stock certificates, and other documents relating to the organization, maintenance, and existence of Seller as a corporation;

(ii) real property;

(iii) Intellectual Property (except as included in the Acquired Assets under **Section 2(a)(v)**);

(iv) franchises, approvals, permits, licenses, orders, registrations, certificates, variances, and similar rights obtained from governments and governmental agencies;

(v) automobiles, trucks, tractors, trailers or tools;

(vi) claims, deposits, prepayments, refunds, causes of actions, choses in action, rights of recovery, rights of set-off, and rights of recoupment (including any such item relating to the payment of Taxes);

(vii) securities; or

(viii) any of the rights of Seller under this Agreement (or under any side agreement between Seller on the one hand and Purchaser on the other hand entered into on or after the date of this Agreement).

(c) *Assumption of Liabilities.* On and subject to the terms and conditions of this Agreement, Purchaser agrees to assume and become responsible for, at the Closing, and to pay and perform in accordance with their terms, the following (the "**Assumed Liabilities**"): (i) all obligations of Seller under the agreements, contracts, leases, licenses, and other arrangements referred to in the definition of Acquired Assets; (ii) all obligations and liabilities relating to any Inventory or Appliance used or made for use in the Business or relating to any services performed as part of the Business by Seller, its employees, agents, subcontractors and successors, on or prior to the Closing Date, whether known or unknown, whether asserted or unasserted, whether absolute or contingent, whether accrued or unaccrued, whether liquidated or unliquidated, and whether due or to become due, including warranty obligations and product liability claims relating to Appliances delivered to or installed on Customer premises; (iii) all

liabilities related to or arising out of Purchaser's actions with respect to the Appliances or the Business in connection with Purchaser's obligations under that certain Letter of Intent between the Purchaser and the Seller dated June 8, 2005; and (iv) obligations after Closing arising from Purchaser's operation of the Business; *provided, however*, that, notwithstanding the above, the Assumed Liabilities shall not include (collectively, the "**Excluded Liabilities**"): (t) any liability of Seller for Income Taxes; (u) any liability relating to payroll, vacation, sick leave, workers' compensation, unemployment benefits, pension benefits, employee stock option or profit-sharing plans, health care plans or benefits or any other employee plans or benefits of any kind for Seller's employees or former employees or both (including any successor liability with respect to any such employee matters); (v) any liability under any employment, severance, retention or termination agreement with any employee of Seller; (w) any liability arising out of or relating to any employee grievance for matters occurring prior to Closing, whether or not the affected employees are hired by Purchaser; (x) any liability of Seller for costs and expenses incurred in connection with this Agreement and the transactions contemplated hereby, or (y) any liability or obligation of Seller under this Agreement (or under any side agreement between Seller on the one hand and Purchaser on the other hand entered into on or after the date of this Agreement).

(d) *Cash Consideration.* Purchaser agrees to pay to Seller at the Closing $6,500,000 by delivery of cash payable by wire transfer or delivery of other immediately available funds.

(e) *The Closing.* The closing of the transactions contemplated by this Agreement (the "**Closing**") shall take place at the offices of Kilpatrick Stockton LLP on the third business day following the satisfaction or waiver of all conditions to the obligations of the Parties to consummate the transactions contemplated hereby (other than conditions with respect to actions the respective Parties will take at the Closing itself) or such other date as the Parties may mutually determine (the "**Closing Date**").

(f) *Deliveries at the Closing.* At the Closing:

(i) Seller will deliver to Purchaser:

a. A certificate, signed by the Secretary of Seller, attaching Seller's: (x) certificate of incorporation; (y) bylaws; and (z) resolutions authorizing the transactions contemplated by this Agreement; and

b. A Transition Services Agreement, in the form of **Exhibit A**, executed by Seller.

(ii) Purchaser will deliver to Seller:

a. the consideration specified in **Section 2(d)**;

b. A certificate, signed by the Secretary of Purchaser, attaching Purchaser's: (x) certificate of incorporation; (y) bylaws; and (z) resolutions authorizing the transactions contemplated by this Agreement; and

c. A Transition Services Agreement, in the form of **Exhibit A**, executed by Purchaser.

(g) *Allocation*. The Parties agree to allocate the consideration set forth in **Section 2(d)** (and all other capitalizable costs) for all purposes (including financial accounting and Tax purposes) in accordance with the allocation schedule attached hereto as **Exhibit B**.

Section 3. *Seller's and AGL's Representations and Warranties.*

Seller and AGL jointly and severally represent and warrant to Purchaser as follows:

(a) *Organization of Seller*. Each of AGL and Seller is a corporation duly organized, validly existing, and in good standing under the laws of the jurisdiction of its incorporation.

(b) *Authorization of Transaction*. Each of AGL and Seller has full power and authority (including full corporate power and authority) to execute and deliver this Agreement and to perform its obligations hereunder. Assuming valid authorization, execution and delivery of this Agreement by Purchaser, this Agreement constitutes the valid and legally binding obligation of each of Seller and AGL, enforceable in accordance with its terms and conditions, except as may be limited by the applicable laws of bankruptcy, insolvency, reorganization and moratorium and other similar laws relating to creditors' rights generally and to general principles of equity.

(c) *Non-contravention*. Except as set forth on **Schedule 3(c)**, neither the execution and delivery of this Agreement, nor the consummation of the transactions contemplated hereby, will (i) violate any constitution, statute, regulation, rule, injunction, judgment, order, decree, ruling, charge, or other restriction of any government, governmental agency, or court to which Seller or AGL is subject or any provision of the certificate of incorporation or bylaws of Seller or AGL or (ii) conflict with, result in a breach of, constitute a default under, result in the acceleration of, create in any party the right to accelerate, terminate, or require any notice under any agreement, contract, lease, license, instrument, or other arrangement to which Seller or AGL is a party or by which it is bound or to which any of the Acquired Assets is subject (or result in the imposition of any Lien upon any of the Acquired Assets), except where the violation, conflict, breach, default, acceleration, termination, modification, cancellation, failure to give notice, or Lien would not have a Material Adverse Effect. Except as set forth on **Schedule 3(c)**, neither Seller nor AGL needs to give any notice to, make any filing with, or obtain any authorization, consent, or approval of any government or governmental agency in order for the Seller or AGL to perform its obligations hereunder, except where the failure to give notice, to file, or to obtain any authorization, consent, or approval would not have a Material Adverse Effect.

(d) *Brokers' Fees*. Neither Seller nor AGL has any liability or obligation to pay any fees or commissions to any broker, finder, or agent with respect to the transactions contemplated by this Agreement for which Purchaser could become liable or obligated.

(e) *Title to Assets*. Seller has marketable title to, or a valid leasehold interest in, the Acquired Assets, free and clear of any Liens or restriction on transfer.

(f) *Financial Statements.* Attached hereto as **Schedule 3(f)** are the following financial statements of Seller (collectively the "**Financial Statements**"): (i) audited consolidated balance sheets as of and for the fiscal years ended December 31, 2003 and December 31, 2004 (the "**Most Recent Fiscal Year End**"); and (ii) unaudited consolidated balance sheet (the "**Most Recent Financial Statements**") as of and for the six months ended June 30, 2005 (the "**Most Recent Fiscal Month End**"). The Financial Statements (including the notes thereto) have been prepared in accordance with GAAP throughout the periods covered thereby, present fairly in all material respects the financial condition of Seller as of such dates and the results of operations of Seller for such periods; *provided, however*, that the Most Recent Financial Statements are subject to normal year-end adjustments and lack footnotes and other presentation items.

(g) *Events Subsequent to Most Recent Fiscal Year End.* Since the Most Recent Fiscal Year End, there has not been any Material Adverse Change. Without limiting the generality of the foregoing, except as set forth on **Schedule 3(g)**, since that date and solely with respect to the Business:

(i) Seller has not entered into any material lease outside the Ordinary Course of Business;

(ii) no party (including Seller) has accelerated, terminated, made material modifications to, or cancelled any material lease to which Seller is a party or by which Seller is bound;

(iii) no material supplier of the Business has indicated, in writing, that it shall stop, or materially decrease the rate of, supplying materials, products or services to the Business;

(iv) Seller has not imposed any Lien upon any of the Acquired Assets;

(v) Seller has not experienced any damage, destruction, or loss (whether or not covered by insurance) to any material portion of the Acquired Assets;

(vi) Seller has not changed its normal business practices or taken any other action outside the Ordinary Course of Business in order to generate Cash; and

(vii) Seller has not committed to any of the foregoing.

(h) *Legal Compliance.* Except as set forth on **Schedule 3(h)**, Seller has, in its operation of the Business, complied with all applicable laws (including rules, regulations, codes, plans, injunctions, judgments, orders, decrees, rulings, and charges thereunder of federal, state, or local governments (and all agencies thereof) and no action, suit, proceeding, hearing, investigation, charge, complaint, claim, demand, or notice has been filed or commenced alleging any failure so to comply, except where the failure to comply would not have a Material Adverse Effect.

(i) *Tax Matters:*

(i) Seller has filed all Income Tax Returns that it was required to file. All such Income Tax Returns were correct and complete in all material respects. All income Taxes owed by Seller (whether or not shown on any Tax Return) have been paid, except where the failure to so pay would not have a Material Adverse Affect. With respect to the Business, Seller has withheld and paid all Taxes required to have been withheld and paid in connection with amounts paid or owing to any employee, independent contractor, creditor, stockholder, or other third party, and all Forms W-2 and 1099 required with respect thereto have been properly completed and timely filed, except where the failure to so pay would not have a Material Adverse Effect.

(ii) The unpaid Taxes of Seller with respect to the Business: (A) did not, as of the Most Recent Fiscal Month End, exceed the reserve for Tax liability set forth in the balance sheet contained within the Most Recent Financial Statements (the "**Most Recent Balance Sheet**"); and (B) will not exceed that reserve as adjusted for operations and transactions through the Closing Date in accordance with the past custom and practice of Seller in filing its Tax Returns.

(iii) There is no dispute or claim concerning any Tax Liability affecting the Acquired Assets either (A) claimed or raised by any authority in writing or (B) as to which any of the directors or officers (or employees responsible for Tax matters) of Seller has Knowledge.

(j) *Inventory*. To Seller's Knowledge, the Inventory is merchantable and fit for the purpose for which it was procured or manufactured and is not slow-moving, obsolete, damaged, or defective, subject to: (i) normal wear and tear; and (ii) the reserve for inventory writedown set forth in the Most Recent Balance Sheet as adjusted for operations and transactions through the Closing Date.

(k) *Contracts*. **Schedule 3(k)** lists, with respect to the Business, the following:

(i) any agreement (or group of related agreements) for the purchase of materials, supplies, goods, services, equipment or other assets;

(ii) any agreement (or group of related agreements) under which Seller has imposed a Lien on any of the Acquired Assets;

(iii) any agreement under which the consequences of a default or termination could have a Material Adverse Effect;

(iv) any agreement concerning a partnership or joint venture;

(v) any agreement (or group of related agreements) under which it has created, incurred, assumed, or guaranteed any indebtedness for borrowed money, or any capitalized lease obligation, in excess of $25,000 or under which it has imposed a Security Interest on any of its assets, tangible or intangible.

Seller has either delivered to Purchaser a correct and complete copy of each written agreement or provided Purchaser access to inspect each written agreement listed on **Schedule 3(k)**. **Schedule**

3(k) contains a written summary of each oral agreement setting forth the terms and conditions of each agreement required to be listed herein. Except as set forth on **Schedule 3(k)**, with respect to each such agreement: (A) the agreement is legal, valid, binding, enforceable, and in full force and effect in all material respects, except as may be limited by the applicable laws of bankruptcy, insolvency, reorganization and moratorium and other similar laws relating to creditors' rights generally and to general principles of equity and will continue to be legal, valid binding and enforceable and in full force and effect in all material respects following the consummation of the transaction contemplated hereby; (B) Seller is not in material breach or default and no event has occurred which with notice or lapse of time would constitute a breach or default or permit the termination, modification or acceleration under the agreement; and (C) no party has repudiated any material provision of the agreement.

(l) *Notes and Accounts Receivable.* All notes and accounts receivable of the Business are reflected properly on its books and records and arose from bona fide transactions conducted in the Ordinary Course of Business.

(m) *Product Liability.* Except as set forth on **Schedule 3(m)**, there is no pending or to Seller's Knowledge, threatened claim in writing asserting any material liability against Seller or the Business arising out of any injury to individuals or property as a result of the ownership of, possession of, use of, maintenance of, delivery of or any other service performed with respect to, any Appliance sold or leased by Seller.

(n) *Customers.* **Schedule 3(n)** lists those Customers from whom the Business has derived revenue during the past twelve (12) months. Except as set forth on **Schedule 3(n)**, to Seller's Knowledge, there are no pending or threatened disputes of a material nature with any Customers or group of Customers.

(o) *Powers of Attorney.* There are no outstanding powers of attorney executed on behalf of the Seller or that affect the Acquired Assets.

(p) *Litigation.* **Schedule 3(p)** sets forth each instance in which the Seller (i) is subject to any outstanding injunction, judgment, order, decree, ruling, or charge or (ii) is a party or is threatened to be made a party to any action, suit, proceeding, hearing, or investigation of, in, or before any court or quasi-judicial or administrative agency of any federal, state, local, or foreign jurisdiction or before any arbitrator. None of the actions, suits, proceedings, hearings, and investigations set forth in **Schedule 3(p)** could result in any Material Adverse Change in the business, financial condition, operations, results of operations, or future prospects of the Seller. Neither the Seller nor any of its directors or officers (or employees with responsibility for litigation matters) has any reason to believe that any such action, suit, proceeding, hearing or investigation may be brought or threatened against the Seller.

(q) *Material Licenses and Permits.* **Schedule 3(q)** attached hereto describes every license, permit, registration and governmental approval with governmental authorities entered into by Seller, which is in effect or has been applied for or is pending (the "**Permits**").

Section 4. *Purchaser's Representations and Warranties.*

Purchaser represents and warrants to Seller as follows:

(a) *Organization of Purchaser.* Purchaser is a limited liability company duly organized, validly existing, and in good standing under the laws of the jurisdiction of its formation.

(b) *Authorization of Transaction.* Purchaser has full power and authority (including full limited liability company power and authority) to execute and deliver this Agreement and to perform its obligations hereunder. Assuming valid authorization, execution and delivery of this Agreement by Seller, this Agreement constitutes the valid and legally binding obligation of Purchaser, enforceable in accordance with its terms and conditions, except as may be limited by the applicable laws of bankruptcy, insolvency, reorganization and moratorium and other similar laws relating to creditors' rights generally and to general principles of equity. Purchaser need not give any notice to, make any filing with, or obtain any authorization, consent, or approval of any government or governmental agency in order to consummate the transactions contemplated by this Agreement. Purchaser has duly authorized the execution, delivery and performance of this Agreement and all other agreements contemplated hereby.

(c) *Non-contravention.* Neither the execution and delivery of this Agreement, nor the consummation of the transactions contemplated hereby, will (i) violate any constitution, statute, regulation, rule, injunction, judgment, order, decree, ruling, charge, or other restriction of any government, governmental agency, or court to which Purchaser is subject or any provision of its certificate of incorporation, or other governing documents or (ii) conflict with, result in a breach of, constitute a default under, result in the acceleration of, create in any party the right to accelerate, terminate, modify, or cancel, or require any notice under any agreement, contract, lease, license, instrument, or other arrangement to which Purchaser is a party or by which it is bound or to which any of its assets are subject. Purchaser does not need to give any notice to, make any filing with, or obtain any authorization, consent, or approval of any government or governmental agency in order for the Parties to consummate the transactions contemplated by this Agreement.

(d) *Brokers' Fees.* Purchaser has no liability or obligation to pay any fees or commissions to any broker, finder, or agent with respect to the transactions contemplated by this Agreement for which Seller could become liable or obligated.

Section 5. *Post-Closing Covenants.*

(a) *[Non-Competition.*

(i) Purchaser covenants and agrees with Seller that for a period of ten (10) years following the Closing Date (the "**Restricted Period**"), Purchaser shall not, and shall cause each of its Affiliates not to, engage, directly or indirectly, as an owner or partner, through stock ownership, investment of capital, lending of money or property, rendering of services, or otherwise, either alone or in association with others, in the operation, management or supervision of any type of business or enterprise that leases or services electric water heaters, electric ranges or electric clothes dryers in those cities in which Pivotal Utility Holdings, Inc. (d/b/a Florida City Gas) provides gas services, as listed on **Schedule 5(a)**; *provided*, (a) that Purchaser may own shares in a publicly-traded corporation or publicly-traded mutual fund or publicly-traded limited partnership in

which Purchaser does not materially participate and in which Purchaser's ownership interest is five percent (5%) or less; and (b) that Purchaser and its Affiliates may lease electric water heaters, electric ranges and electric clothes dryers in such cities, so long as: (1) Purchaser does not lease electric heaters, electric ranges and electric clothes dryers unless a customer of the Business specifically requests electric heaters, electric ranges or electric clothes dryers, and (2) the total number of electric heaters, electric ranges and electric clothes dryers leased by Purchaser in the locations set forth on **Schedule 5(a)** do not exceed two percent (2%) of all heaters, ranges and clothes dryers leased by Purchaser at those locations (set forth on **Schedule 5(a)**) capable of using gas heaters, gas ranges or gas clothes dryers.]

(ii) In the event that any court determines that the duration or the geographic scope, or both, of the provision set forth in **Section 5(a)(i)** are unreasonable and that such provision is to that extent unenforceable, the Parties hereto agree that the provision set forth in **Section 5(a)(i)** shall remain in full force and effect for the greatest time period and in the greatest areas that would not render it unenforceable. The Parties intend that this non-competition provision shall be deemed to be a series of separate covenants, one for each and every county of Florida. Purchaser agrees that Seller shall, whether or not it is pursuing any potential remedies at law, be entitled to equitable relief in the form of preliminary and permanent injunctions without bond or other security upon any actual or threatened breach of this non-competition provision.

(b) *Delivery of Inventory*. From the period beginning the day after the Closing Date and continuing for ten (10) days thereafter, Seller shall make the Inventory available at Seller's location(s) set forth on **Schedule 5(b)**. Prior to the expiration of such period, Purchaser shall, at its own cost and expense, take possession of the Inventory and remove the Inventory from Seller's premises; provided, that Seller shall allow the Inventory to remain at Seller's location(s) set forth on **Schedule 5(b)** until November 30, 2005 so long as: (i) Purchaser acknowledges and agrees that Seller shall have abandoned such properties within ten (10) days after the Closing Date and shall not have any obligations or liabilities with respect to such location(s) or the Inventory kept therein after such date (including any obligation to maintain any form of insurance, security, etc.); and (ii) Purchaser agrees to indemnify and hold harmless Seller against any and all Adverse Consequences incurred by AGL or Seller for any and all actions taken or not taken by Purchaser or its Affiliates (including, but not limited to its and its Affiliates' officers, directors, employees, representatives, agents or third-party contractors) with respect the Inventory and such location(s) after such date. Notwithstanding the foregoing, to the extent the Inventory remains at such location(s) past such ten (10) day period, Seller agrees to inspect such Inventory for apparent defects and provide reasonably prompt notice to Purchaser regarding any such defects; provided, that Seller shall have no additional obligation or liability with respect to the Inventory beyond conducting such inspection and providing such notice. Seller agrees to reasonably cooperate with Purchaser in order for Purchaser to perform its obligations under this **Section 5(b)**, including by granting Purchaser's agents reasonable access to those locations set forth on **Schedule 5(b)**.

(c) *No License*. Nothing herein conveys any license (express or implied) to Purchaser to use the name, trademark or logo of Seller or its parent or Affiliates. Any such use

of the mark is strictly prohibited without prior written consent and may require the execution of a trademark license agreement.

Section 6. Remedies for Breaches of This Agreement.

(a) *Survival of Representations and Warranties.* Except for the representations and warranties of Seller and Purchaser, as applicable, contained in **Sections 3(a), 3(b), 3(d), 3(e), 4(a), 4(b)** and **4(d)**, which shall survive the Closing and continue in full force and effect indefinitely, and except for the representations, warranties and covenants of Seller and Purchaser, as applicable, contained in **Sections 3(i), 5(a)** and **5(b)**, each of which shall survive the Closing and continue in full force and effect until the expiration of any applicable statute of limitation (after giving effect to any extensions or waivers), all of the representations and warranties of Seller contained in **Section 3** shall survive the Closing and continue in full force and effect solely for a period of fifteen (15) months thereafter.

(b) *Indemnification Provisions for Purchaser's Benefit.*

(i) In the event Seller or AGL breaches (or in the event any third party alleges facts that, if true, would mean Seller or AGL has breached) any of its representations, warranties, and covenants contained in this Agreement, and provided that Purchaser makes a written claim for indemnification against Seller within the applicable survival period, then Seller and AGL, jointly and severally, shall indemnify Purchaser from and against the entirety of any Adverse Consequences Purchaser may suffer resulting from, arising out of, relating to, in the nature of, or caused by the breach (or the alleged breach); provided, however, that Seller shall not have any obligation to indemnify Purchaser from and against any Adverse Consequences resulting from, arising out of, relating to, in the nature of, or caused by the breach (or alleged breach) of any representation or warranty of Seller contained in **Section 3(c)** and **Section 3(f)** through **Section (o)** unless and until Purchaser has suffered Adverse Consequences by reason of all such breaches (or alleged breaches) in excess of $75,000, at which point Seller will be obligated to indemnify Purchaser from and against all Adverse Consequences in excess of such threshold.

(ii) In addition to Seller's and AGL's obligations contained in **Section 6(b)(i)**, Seller and AGL shall jointly and severally indemnify Purchaser from and against the entirety of any Adverse Consequences Purchaser may suffer resulting from, arising out of, relating to, in the nature of, or caused by any Excluded Liability.

(c) *Indemnification Provisions for Seller's Benefit.*

(i) In the event Purchaser breaches (or in the event any third party alleges facts that, if true, would mean Purchaser has breached) any of its representations, warranties, and covenants contained in this Agreement and, provided that Seller makes a written claim for indemnification against Purchaser within the applicable survival period, then Purchaser shall indemnify Seller from and against the entirety of any Adverse Consequences Seller may suffer resulting from, arising out of, relating to, in the nature of, or caused by the breach (or the alleged breach).

(ii) Purchaser agrees to indemnify Seller from and against the entirety of any Adverse Consequences Seller may suffer resulting from, arising out of, relating to, in the nature of, or caused by any Assumed Liability.

(iii) Purchaser agrees to indemnify Seller from and against the entirety of any Adverse Consequences Seller may suffer resulting from, arising out of, relating to, in the nature of, or caused by Purchaser's breach of its obligations under Section 5.

(d) *Matters Involving Third Parties.*

(i) If any third party notifies either Party (the "**Indemnified Party**") with respect to any matter (a "**Third-Party Claim**") that may give rise to a claim for indemnification against the other Party (the "**Indemnifying Party**") under this **Section 6**, then the Indemnified Party shall promptly notify the Indemnifying Party thereof in writing; *provided, however*, that no delay on the part of the Indemnified Party in notifying the Indemnifying Party shall relieve the Indemnifying Party from any obligation hereunder to the extent the Indemnifying Party is materially prejudiced thereby.

(ii) The Indemnifying Party will have the right to defend the Indemnified Party against the Third-Party Claim with counsel of its choice reasonably satisfactory to the Indemnified Party so long as (A) the Indemnifying Party notifies the Indemnified Party in writing within fifteen (15) days after the Indemnified Party has given notice of the Third-Party Claim that the Indemnifying Party will indemnify the Indemnified Party from and against the entirety of any Adverse Consequences the Indemnified Party may suffer resulting from, arising out of, relating to, in the nature of, or caused by the Third-Party Claim, and (B) the Indemnifying Party conducts the defense of the Third-Party Claim actively and diligently.

(iii) So long as the Indemnifying Party is conducting the defense of the Third-Party Claim in accordance with **Section 6(d)(ii)**, (A) the Indemnified Party may retain separate co-counsel at its sole cost and expense and participate in the defense of the Third-Party Claim, (B) the Indemnified Party will not consent to the entry of any judgment or enter into any settlement with respect to the Third-Party Claim without the prior written consent of the Indemnifying Party (not to be unreasonably withheld), and (C) the Indemnifying Party will not consent to the entry of any judgment or enter into any settlement with respect to the Third-Party Claim without the prior written consent of the Indemnified Party (not to be unreasonably withheld); provided, that, the Indemnified Party's consent need not be obtained so long as: (y) the relief provided is monetary damages that are less than the maximum amount set forth in **Section 6(e)** or **(f)**, as applicable, and is paid in full by the Indemnifying Party or (z) the relief consists of an injunction or other equitable relief which does not directly impact the Indemnified Party.

(iv) If any of the conditions in **Section 6(d)(ii)** is or becomes unsatisfied, then (A) the Indemnified Party may defend against, and consent to the entry of any judgment on or enter into any settlement with respect to, the Third-Party Claim in any manner it may reasonably deem appropriate and the Indemnified Party need not consult with, or obtain any consent from, the Indemnifying Party in connection therewith, (B) the

Indemnifying Party will reimburse the Indemnified Party promptly and periodically for the reasonable costs of defending against the Third-Party Claim, including reasonable attorneys' fees and expenses, and (C) the Indemnifying Party will remain responsible for any Adverse Consequences the Indemnified Party may suffer resulting from, arising out of, relating to, in the nature of, or caused by the Third-Party Claim to the fullest extent provided in this **Section 6**.

(e) *Maximum Indemnification Obligations.* Notwithstanding the foregoing, in addition to the limitations set forth in **Sections 6(f)** and **6(g)**, in no event shall either Party's obligations under this **Section 6** exceed $650,000. For purposes of clarification, the limitation set forth in this **Section 6(e)** shall be separate and distinct from the limitations set forth in **Sections 6(f)** and **(g)**, and claims paid under **Sections 6(f)** and **(g)** shall not apply against the limitation set forth in this **Section 6(e)**.

(g) *Indemnification Related to the Open Permits.* In addition to the other indemnification provisions in this **Section 6**, Seller agrees to indemnify Purchaser from and against the entirety of any Adverse Consequences Purchaser may suffer resulting from, arising out of, relating to, in the nature of, or caused by any Open Permit. Notwithstanding the foregoing, in no event shall Seller's obligations under this **Section 6(f)** exceed $500,000.

(h) *Indemnification Related to Seller's Installation of Appliances.* In addition to the other indemnification provisions in this **Section 6**, Seller agrees to indemnify Purchaser from and against the entirety of any Adverse Consequences Purchaser may suffer solely and directly resulting from Purchaser's, its employees or its third-party contractor's negligent or improper installation of gas heaters, gas ranges and gas clothes dryers. Notwithstanding the foregoing, Seller's obligations under this **Section 6(g)**: (i) shall not apply to any Adverse Consequences Purchaser suffers on or after the date that is twelve (12) months after the Closing Date; and (ii) shall not, in any event, exceed $1,000,000.

(i) *Exclusive Remedy.* Except with respect to claims involving fraud, willful misconduct or violations of law, the rights to indemnification set forth in this **Section 6** shall be the sole and exclusive remedy of the Parties for any breach of or inaccuracy in any representation or warranty or any breach of any covenant or agreement of the other Party in this Agreement. Each Party hereby foregoes any other rights that it may have for the matters set forth in this **Section 6**, including all statutory, common law and other claims with respect thereto, save for the right to seek injunctive relief for a breach or threatened breach of any covenant or agreement by any Party.

Section 7. Miscellaneous.

(a) *Dispute Resolution.* At the written request of either party, each party will appoint a knowledgeable, responsible representative to meet and negotiate in good faith to resolve any claim, controversy or dispute arising out of or relating to this Agreement. The parties intend that these negotiations be conducted by non-lawyer, business representatives. The location, format, frequency, duration and conclusion of these negotiations shall be left to the discretion of the representatives; provided, that such negotiations shall not exceed, in the aggregate, fifteen (15) days in duration. Discussions and correspondence among the representatives for purposes of

these negotiations shall be treated as confidential information developed for purposes of settlement, exempt from discovery, and shall not be admissible in any lawsuit without the concurrence of all parties. Documents identified in or provided with such communications, which are not prepared for purposes of the negotiations, are not so exempted and may, if otherwise discoverable or admissible, be discovered, or be admitted in evidence in the arbitration or lawsuit. At the conclusion of such fifteen (15) day period, either party may exercise any other remedy, whether equitable or otherwise, available to it under applicable law. Notwithstanding the foregoing, nothing in this dispute resolution provision shall prevent a party from seeking immediate interim relief from an appropriate court in appropriate circumstances.

(b) *Press Releases and Public Announcements.* No Party shall issue any press release or make any public announcement relating to the subject matter of this Agreement prior to the Closing without the prior written approval of the other Party, not to be unreasonably withheld; *provided, however*, that any Party may make any public disclosure it believes in good faith is required by applicable law or any listing or trading agreement concerning its publicly traded securities (in which case the disclosing Party will use its reasonable efforts to advise the other Party prior to making the disclosure).

(c) *No Third-Party Beneficiaries.* This Agreement shall not confer any rights or remedies upon any Person other than the Parties and their respective successors and permitted assigns.

(d) *Entire Agreement.* This Agreement (including the documents referred to herein) constitutes the entire agreement between the Parties with respect to the subject matter hereof and supersedes any prior understandings, agreements, or representations by or between the Parties, written or oral, to the extent they relate in any way to the subject matter hereof.

(e) *Succession and Assignment.* This Agreement shall be binding upon and inure to the benefit of the Parties named herein and their respective successors and permitted assigns. No Party may assign either this Agreement or any of its rights, interests, or obligations hereunder without the prior written approval of the other Party, except that Purchaser may assign this Agreement to an Affiliate.

(f) *Counterparts.* This Agreement may be executed in one or more counterparts (including by means of facsimile), each of which shall be deemed an original but all of which together will constitute one and the same instrument.

(g) *Headings.* The section headings contained in this Agreement are inserted for convenience only and shall not affect in any way the meaning or interpretation of this Agreement.

(h) *Notices.* All notices, requests, demands, claims, and other communications hereunder shall be in writing. Any notice, request, demand, claim, or other communication hereunder shall be deemed duly given (i) when delivered personally to the recipient, (ii) one (1) business day after being sent to the recipient by reputable overnight courier service (charges prepaid), (iii) one (1) business day after being sent to the recipient by facsimile transmission or electronic mail, or (iv) four (4) business days after being mailed to the recipient by certified or

registered mail, return receipt requested and postage prepaid, and addressed to the intended recipient as set forth below:

If to Seller: NUI Service, Inc.
955 E 25th Street
Hialeah, FL 33013
Attention: Charles Rawson
Fax:

With a copy to (which shall not constitute notice):
AGL Resources
Ten Peachtree Place, 15th Floor
Atlanta, GA 30309
Attention: General Counsel
Fax: (404) 584-3714

With a copy to (which shall not constitute notice):
Kilpatrick Stockton LLP
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309
Attention: Jim Steinberg, Esq.
Fax: (404) 815-6555

If to Purchaser: AWHR America's Water Heater Rentals, LLC
12470 Olive Blvd.
St. Louis, Missouri 63141
Attention:
Fax:

With a copy to (which shall not constitute notice):
Armstrong Teasdale LLP
One Metropolitan Square, Suite 2600
St. Louis, Missouri 63102
Attention: Steven E. Pozaric, Esq.
Fax: (314) 612-2343

Any Party may change the address to which notices, requests, demands, claims, and other communications hereunder are to be delivered by giving the other Party notice in the manner set forth herein.

(i) *Governing Law.* This Agreement shall be governed by and construed in accordance with the laws of the State of Georgia without giving effect to any choice or conflict of law provision or rule (whether of the State of Georgia or any other jurisdiction) that would cause the application of the laws of any jurisdiction other than the State of Georgia.

(j) *Amendments and Waivers.* No amendment of any provision of this Agreement shall be valid unless the same shall be in writing and signed by Purchaser and Seller. No waiver

by any Party of any provision of the Agreement or any default, misrepresentation, or breach of warranty or covenant hereunder, whether intentional or not, shall be valid unless the same shall be in writing and signed by the Party making such waiver nor shall such waiver be deemed to extend to any prior or subsequent default, misrepresentation, or breach of warranty or covenant hereunder or affect in any way any rights arising by virtue of any prior or subsequent such occurrence.

(k) *Severability*. In the event that any court of competent jurisdiction shall finally determine that any provision, or any portion thereof, contained in this Agreement shall be void, unreasonable or unenforceable in any respect, then such provision shall be deemed limited to the extent that such court determines it enforceable or reasonable, and as so limited shall remain in full force and effect. In this regard, the Parties agree that any judicial authority construing this Agreement shall be empowered to sever, reform and/or blue-pencil any such unenforceable provision or portion thereof to make such provision or portion thereof and/or this Agreement remain enforceable to the fullest extent allowed by applicable law and to protect the proper interests of the Parties in accomplishing the purposes of the covenants contained herein.

(l) *Expenses*. Each of Purchaser and Seller will bear its own costs and expenses (including legal fees and expenses) incurred in connection with this Agreement and the transactions contemplated hereby; *provided, however*, that all transfer, documentary, sales, use, stamp, registration and other such Taxes, and all conveyance fees, recording charges and other fees and charges (including any penalties and interest) incurred in connection with the consummation of the transactions contemplated by this Agreement shall be borne by Purchaser.

(m) *Construction*. The Parties have participated jointly in the negotiation and drafting of this Agreement. If an ambiguity or question of intent or interpretation arises, then this Agreement shall be construed as if drafted jointly by the Parties and no presumption or burden of proof shall arise favoring or disfavoring any Party by virtue of the authorship of any of the provisions of this Agreement. Any reference to any federal, state or local statute or law shall be deemed also to refer to all rules and regulations promulgated thereunder, unless the context requires otherwise. The word *including* shall mean including without limitation.

(n) *Incorporation of Exhibits and Schedules*. The Exhibits and Schedules identified in this Agreement are incorporated herein by reference and made a part hereof.

(o) *Bulk Transfer Laws*. Purchaser acknowledges that Seller will not comply with the provisions of any bulk transfer laws of any jurisdiction in connection with the transactions contemplated by this Agreement.

[Remainder of Page Intentionally Left Blank]

IN WITNESS WHEREOF, the Parties hereto have executed this Agreement as of the date first above written.

AMERICA'S WATER HEATER RENTALS, LLC

By: _____

Title: _____

NUI SERVICE, INC.

By: _____
Title: _____

AGL RESOURCES, INC.

By: _____
Its: _____

ANNEX I
DEFINITIONS

"*Acquired Assets*" has the meaning set forth in **Section 2(a)**.

"*Adverse Consequences*" means all actions, suits, proceedings, hearings, investigations, charges, complaints, claims, demands, injunctions, judgments, orders, decrees, rulings, damages, dues, penalties, fines, costs, amounts paid in settlement, Liabilities, obligations, Taxes, Liens, losses, expenses, and fees, including court costs and attorneys' fees and expenses.

"*Affiliate*" has the meaning set forth in Rule 12b-2 of the regulations promulgated under the Securities Exchange Act.

"*AGL*" has the meaning set forth in the recitals.

"*Appliances*" has the meaning set forth in the recitals hereto.

"*Assumed Liabilities*" has the meaning set forth in **Section 2(c)**.

"*Business*" has the meaning set forth in the recitals hereto.

"*Cash*" means cash and cash equivalents (including marketable securities and short-term investments) calculated in accordance with GAAP applied on a basis consistent with the preparation of the Financial Statements.

"*Closing*" has the meaning set forth in **Section 2(e)**.

"*Closing Date*" has the meaning set forth in **Section 2(e)**.

"*Code*" means the Internal Revenue Code of 1986, as amended.

"*Customer*" has the meaning set forth in **Section 2(a)(i)**.

"*Excluded Liability*" has the meaning set forth in **Section 2(c)**.

"*Excluded Assets*" has the meaning set forth in **Section 2(b)**.

"*Financial Statements*" has the meaning set forth in **Section 3(f)**.

"*GAAP*" means United States generally accepted accounting principles in effect from time to time, consistently applied.

"*Income Tax*" means any federal, state or local income tax, including any interest, penalty, or addition thereto, whether disputed or not.

"*Income Tax Return*" means any return, declaration, report, claim for refund, or information return or statement relating to Income Taxes, including any schedule or attachment thereto, and including any amendment thereof.

"*Indemnified Party*" has the meaning set forth in **Section 6(d)**.

"*Indemnifying Party*" has the meaning set forth in **Section 6(d)**.

"*Intellectual Property*" means all of the following in any jurisdiction throughout the world: (i) all inventions (whether patentable or unpatentable and whether or not reduced to practice), all improvements thereto, and all patents, patent applications, and patent disclosures, together with all reissuances, continuations, continuations-in-part, revisions, extensions, and reexaminations thereof, (ii) all trademarks, service marks, trade dress, logos, slogans, trade names, corporate names, Internet domain names, and rights in telephone numbers, together with all translations, adaptations, derivations, and combinations thereof and including all goodwill associated therewith, and all applications, registrations, and renewals in connection therewith, (iii) all copyrightable works, all copyrights, and all applications, registrations, and renewals in connection therewith, (iv) all mask works and all applications, registrations, and renewals in connection therewith, (v) all trade secrets and confidential business information (including ideas, research and development, know-how, formulas, compositions, manufacturing and production processes and techniques, technical data, designs, drawings, specifications, customer and supplier lists, pricing and cost information, and business and marketing plans and proposals), (vi) all computer software (including source code, executable code, data, databases, and related documentation), (vii) all material advertising and promotional materials, (viii) all other proprietary rights, and (ix) all copies and tangible embodiments thereof (in whatever form or medium).

"*Inventory*" has the meaning set forth in **Section 2(a)(i)**.

"*Knowledge*" an individual will be deemed to have "Knowledge" of a particular fact or other matter if such individual is or was actually aware of such fact or other matter; provided, that an individual will be deemed to have "Knowledge" of a particular fact or other matter if a prudent individual could be expected to discover or otherwise become aware of such fact or matter in the course of reasonably performing their duties.

"*Liability*" means any liability (whether known or unknown, whether asserted or unasserted, whether absolute or contingent, whether accrued or unaccrued, whether liquidated or unliquidated, and whether due or to become due), including any liability for gift certificates or Taxes.

"*Lien*" means any mortgage, pledge, lien, encumbrance, charge, or other security interest other than (i) mechanics', materialmen's, and similar liens, (ii) liens for Taxes not yet due and payable or for Taxes that the taxpayer is contesting in good faith through appropriate proceedings, (iii) purchase money liens and liens securing rental payments under capital lease arrangements, and (iv) other liens arising in the Ordinary Course of Business and not incurred in connection with the borrowing of money.

"*Material Adverse Effect*" or "*Material Adverse Change*" means any effect or change that would be materially adverse to the Acquired Assets, or to the ability of either Party to consummate timely the transactions contemplated hereby; provided, that none of the following shall be deemed to constitute, and none of the following shall be taken into account in determining whether there has been, a Material Adverse Effect or Material Adverse Change: (i) any adverse change, event, development, or effect arising from or relating to (a) general business or economic conditions, including such conditions related to the Business, (b) financial, banking or securities markets (including any disruption thereof or any decline in the price of any security or any market index), (c) changes in GAAP, (d) changes in laws, rules, regulations, orders, or other binding directives issued by any governmental entity or (e) the taking of any action contemplated by this Agreement and the other agreements contemplated hereby, (ii) any existing event, occurrence, or circumstance with respect to which Purchaser has knowledge as of the date hereof and (iii) any adverse change in or effect on the Acquired Assets that is cured by Seller before the Closing Date.

"*Most Recent Balance Sheet*" has the meaning set forth in **Section 3(i)**.

"*Most Recent Financial Statements*" has the meaning set forth in **Section 3(f)**.

"*Most Recent Fiscal Month End*" has the meaning set forth in **Section 3(f)**.

"*Most Recent Fiscal Year End*" has the meaning set forth in **Section 3(f)**.

"*Open Permits*" means those permits described on **Schedule 3(h)**.

"*Ordinary Course of Business*" means the ordinary course of business consistent with past custom and practice (including with respect to quantity and frequency).

"*Party*" has the meaning set forth in the preface hereto.

"*Person*" means an individual, a partnership, a corporation, a limited liability company, an association, a joint stock company, a trust, a joint venture, an unincorporated organization, any other business entity or a governmental entity (or any department, agency, or political subdivision thereof).

"*Purchaser*" has the meaning set forth in the preface hereto.

"*Restricted Customer*" has the meaning set forth in **Section 5(a)(i)**.

"*Restricted Period*" has the meaning set forth in **Section 5(a)(i)**.

"*Securities Exchange Act*" means the Securities Exchange Act of 1934, as amended.

"*Subsidiary*" means, with respect to any Person, any corporation, limited liability company, partnership, association, or other business entity of which (i) if a corporation, a majority of the total voting power of shares of stock entitled (without regard to the occurrence of any contingency) to vote in the election of directors, managers, or trustees thereof is at the time owned or controlled, directly or indirectly, by that Person or one or more of the other

Subsidiaries of that Person or a combination thereof or (ii) if a limited liability company, partnership, association, or other business entity (other than a corporation), a majority of the partnership or other similar ownership interests thereof is at the time owned or controlled, directly or indirectly, by that Person or one or more Subsidiaries of that Person or a combination thereof and for this purpose, a Person or Persons own a majority ownership interest in such a business entity (other than a corporation) if such Person or Persons shall be allocated a majority of such business entity's gains or losses or shall be or control any managing director or general partner of such business entity (other than a corporation). The term "Subsidiary" shall include all Subsidiaries of such Subsidiary.

"*Seller*" has the meaning set forth in the preface hereto.

"*Tax*" or "*Taxes*" means any federal, state or local income, gross receipts, license, payroll, employment, excise, severance, stamp, occupation, premium, windfall profits, environmental (including taxes under Code Section 59A), customs duties, capital stock, franchise, profits, withholding, social security (or similar), unemployment, disability, real property, personal property, sales, use, transfer, registration, value added, alternative or add-on minimum, estimated, or other tax of any kind whatsoever, whether computed on a separate or consolidated, unitary or combined basis or in any other manner, including any interest, penalty, or addition thereto, whether disputed or not.

"*Tax Return*" means any return, declaration, report, claim for refund, or information return or statement relating to Taxes, including any schedule or attachment thereto, and including any amendment thereof.

"*Third-Party Claim*" has the meaning set forth in **Section 6(d)**.

Exhibit A
Transition Services Agreement

Exhibit B
Allocation Schedule